

THE IONIC BASIS OF THE RECEPTOR POTENTIAL OF FROG TASTE CELLS INDUCED BY WATER STIMULI

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Summary

The ionic mechanism underlying the receptor potential induced by a deionized water stimulus was studied in frog taste cells with conventional microelectrodes. The taste cells located in the proximal portion of the tongue generated a depolarizing receptor potential which averaged 10 mV in response to stimulation with deionized water. The cell membrane of the water-sensitive taste cell could be divided into the taste-receptive (apical) and basolateral membranes and the cells were classified into two types: Cl^- -dependent and Cl^- -independent. In Cl^- -dependent cells whose input resistance was decreased or unchanged by deionized water, the magnitude of the water-induced depolarization decreased with an increase in concentration of superficial Cl^- in contact with the receptive membrane and with addition of blockers of anion channels (0.1 mmol l^{-1} SITS and 0.1 mmol l^{-1} DIDS) to deionized water. The reversal potential for the depolarization in this type shifted according to the concentration of superficial Cl^- . These properties of the responses were consistent with those of the glossopharyngeal nerve which innervates the taste disc. In Cl^- -independent cells whose input resistance was increased by deionized water, the reversal potential was approximately equal to the equilibrium potential for K^+ at the basolateral membrane. The water-induced response of the glossopharyngeal nerve was decreased to about 60% of the control value by addition of interstitial $2 \text{ mmol l}^{-1} \text{ Ba}^{2+}$. It is concluded that the water-induced receptor potential is produced by Cl^- secretion through the taste-receptive membrane in about 70% of water-sensitive frog taste cells, while it is generated by an inhibition of the resting K^+ conductance of the basolateral membrane in the remaining 30% of the cells.

Introduction

Zotterman (1949) first discovered that application of pure water to the frog tongue induces impulse discharge in the glossopharyngeal nerve lasting a few minutes. The phenomenon is called the water response. The water response has also been found in mammals, fish and insects (Evans and Mellon, 1962; Gordon *et al.* 1959; Liljestrand and Zotterman, 1954; Yoshii and Kurihara, 1983; Zotterman, 1956). Since the ionic

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concentration of normal mucosal fluid on the tongue is about half that of Ringer's solution (Bradley, 1991), the water response can be induced by stimulating the frog tongue with natural fresh water. It is suggested that the water response may cause the mouth-closing reflex, and that the response may help to stabilize the ionic balance of the body fluid (Zotterman, 1949).

Intracellular receptor potentials induced by deionized water in taste cells have been described in frog (Akaike and Sato, 1976; Sato and Beidler, 1975). Furthermore, Sato and Beidler (1975) demonstrated that those taste cells depolarized by deionized water become hyperpolarized in response to hypertonic NaCl solution.

Using the patch-clamp technique it was found that cyclic AMP and ATP added together to the pipette solution elicited a slow depolarization accompanying an increase in input resistance in frog taste cells (Avenet and Lindemann, 1987; Avenet *et al.* 1988; Okada *et al.* 1990). It is thought that the depolarization may be attributed to the inhibition of the resting K^+ conductance produced by phosphorylation. However, it is not clear whether this mechanism contributes to gustatory transduction. *In situ* the taste cells in the tongue epithelia of frog and mouse are depolarized by an intracellular injection of cyclic nucleotides (Okada *et al.* 1987; Tonosaki and Funakoshi, 1988).

The present study was undertaken to clarify the ionic mechanism underlying the generation of the receptor potential induced by deionized water in frog taste cells by separately replacing the superficial and interstitial fluids of the tongue.

A part of this study has been published in abstract form (Okada *et al.* 1988a,b, 1989).

Materials and methods

Preparation

Fifty six bullfrogs (*Rana catesbeiana*) weighing 174–627g were used for the experiments over the course of a year. The animals were anaesthetized with an intraperitoneal injection of 50% urethane–Ringer's solution at a dose of 3 g kg^{-1} bodymass. To prevent spontaneous contraction of the tongue, the hypoglossal nerve and the hyoglossal muscle were cut bilaterally. The tongue was fully extended and the base of the tongue was fixed with steel pins on a cork plate in an experimental chamber.

Recording

The intracellular potential was recorded in single taste cells by inserting a glass capillary microelectrode (20–50 M Ω), filled with 3 mol l^{-1} KCl, into the taste disc of the fungiform papillae scattered on the tongue. A glass capillary (100–150 μm in tip diameter) filled with 3 mol l^{-1} KCl–3% agar was used as an indifferent electrode and was inserted into the musculature of the lower jaw on which the tongue grows. The ionic environment of the indifferent electrode was not changed during the water stimulus. Under resting and water-stimulus conditions, the potential difference recorded using an indifferent electrode located on the tongue surface was equal to that recorded with an indifferent electrode inserted into the musculature of the lower jaw. This indicated the

presence of an electrical shunting pathway between the superficial and interstitial fluids in the tongue because the surface and the inside of the tongue were directly connected at the base of the tongue by steel pins, as mentioned above. Since taste sensitivity of the frog tongue to deionized water is significantly higher at the proximal region than at the apical region (Nomura and Sakada, 1965; Sato *et al.* 1983), the receptor potentials induced by deionized water were recorded only from the proximal region. The amplitude at the end of stimulation was taken as a measure of the response.

The microelectrode was connected to a preamplifier (DPZ-16A, Dia Medical System, Tokyo) and the membrane potential was recorded on a pen recorder. When the input resistance and reversal potential for a depolarizing response in a taste cell were measured, a bridge circuit was employed which allowed simultaneous current injection and membrane potential recording. The bridge was carefully balanced before the penetration of the cell to cancel the resistance of the microelectrode. The intensity of the current injected through the microelectrode was monitored by the potential drop across a 10M resistor. To inject Cl^- into a taste cell, negative current (300 pulses of 0.5s duration at 1.0 nA) was passed into the cell through the microelectrode filled with 3 mol l⁻¹ KCl.

The whole glossopharyngeal nerves of both sides were dissected free from the surrounding connective tissues and were cut near the hyoid bone. The nerve was placed over bipolar silver wires, for recording the impulses, and immersed in liquid paraffin. The neural impulses were amplified with an a.c. amplifier, integrated with a time constant of 0.3s and recorded on a pen recorder. The amplitude at the end of stimulation was used as a measure of the responses.

Solutions

The ionic compositions of the superficial and interstitial fluids of the tongue are listed in Table 1. Furosemide (Sigma) and Na_3VO_4 (Wako) were dissolved in a normal saline solution. The interstitial fluids contained 20mmol l⁻¹ glucose and 5% (w/v) dextran (relative molecular mass about 70000) (Pharmacia).

Deionized water for taste stimulation was prepared with a reagent grade water-making instrument (Millipore). For taste stimulation, NaCl, NaBr, NaSCN, sodium gluconate, KCl, choline chloride, 4-acetamido-4'-isothiocyanostilbene-2,2'-disulphonic acid (SITS, disodium salt, Nakarai) and 4,4'-diisocyanostilbene-2,2'-disulphonic acid (DIDS, disodium salt, Nakarai) were dissolved in deionized water. Diphenylamine-2-carboxylate (DPC, Nakarai) was dissolved in deionized water containing 0.1% dimethyl sulphoxide (DMSO, Sigma).

Application of solutions

During intracellular recording, either an adapting solution of normal saline or a stimulus of deionized water was perfused onto the tongue surface at a rate of 0.1ml s⁻¹. A detailed description of the method for delivering the solution has already been given (Sato and Beidler, 1975). Frog saline solution, which was stocked in a 1l glass bottle, was flowed continuously over the tongue surface using polyethylene tube (inner diameter, 1 mm). A glass tube with an injection port was attached to the polyethylene tube for

Table 1. *Ionic compositions of solutions*

| Constituent (mmol l ⁻¹) | Normal | Na ⁺ -free | K ⁺ -free | Cd ²⁺ | Ba ²⁺ | Cl ⁻ -free | Na ⁺ -free, Cl ⁻ -free |
|--|--------|-----------------------|----------------------|------------------|------------------|-----------------------|---|
| NaCl | 115 | – | 117.5 | 113.5 | 112 | – | – |
| KCl | 2.5 | 2.5 | – | 2.5 | 2.5 | – | – |
| CaCl ₂ | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | – | – |
| Choline chloride | – | 115 | – | – | – | – | – |
| CdCl ₂ | – | – | – | 1 | – | – | – |
| BaCl ₂ | – | – | – | – | 2 | – | – |
| Sodium gluconate | – | – | – | – | – | 115 | – |
| K ₂ SO ₄ | – | – | – | – | – | 1.25 | 1.25 |
| CaSO ₄ | – | – | – | – | – | 1.8 | 1.8 |
| Sucrose | – | – | – | – | – | – | 230 |

All solutions were buffered with 5mmol l⁻¹ Hepes.

The pH of all solutions was adjusted to 7.2 with NaOH or tetramethylammonium hydroxide (TMAOH).

introduction of taste stimuli. The exchange of the normal interstitial fluid with modified solutions was made using the lingual arterial perfusion method described elsewhere (Okada *et al.* 1985). A peristaltic pump was used for the arterial perfusion. A flexible plastic tube was connected to the pump. Perfusate was drained from one end of the tube and pumped into the other end. This end of the tube was connected to the external carotid artery *via* a polyethylene tube (outer diameter, 0.25–0.30mm) whose tip reached the lingual artery. Perfusate through the lingual artery was drained from the lingual vein. When the gustatory neural response to deionized water had been measured, the adapting and stimulating solutions were perfused onto the tongue surface at 0.5ml s⁻¹ through a 10ml syringe.

Experimental procedure

When the effects of test substances added to deionized water were examined, the control and test responses were obtained from the same taste cell or nerve. Because it was technically difficult to hold a stable penetration of a microelectrode in a taste cell for the long time it took to replace the interstitial fluid of the tongue completely, the effects of modified interstitial fluids upon the receptor potential induced by deionized water were evaluated by comparing the control and test responses obtained from different taste cells in the same tongue.

All the experiments were carried out at room temperature (20–25°C).

Results

Depolarization induced by deionized water stimulation

Fig. 1 shows three examples of the receptor potentials induced by deionized water in frog taste cells after the tongue surface had been adapted to the normal saline solution and with the normal interstitial fluid surrounding the taste cells. When the normal saline solution on the tongue surface was exchanged with deionized water as a taste stimulus,

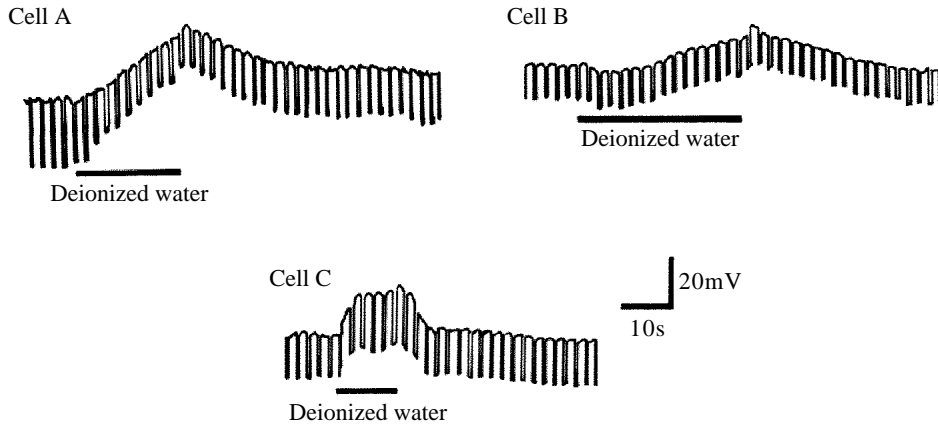


Fig. 1. Three examples of the receptor potentials induced by deionized water in frog taste cells after adaptation of the tongue to normal saline. Each record was obtained from a different taste cell. To monitor the change in input resistance, electrotonic potentials induced by a train of hyperpolarizing current pulses (0.5s duration at 0.5nA) were superimposed on the membrane potential. The resting potentials of the three taste cells were -15mV in cell A, -27mV in cell B and -15mV in cell C. The input resistance of cell A was decreased from 44 to $32\text{M}\Omega$ by deionized water and that of cell C was increased from 34 to $40\text{M}\Omega$ by deionized water. The input resistance of cell B was unchanged.

taste cells produced a depolarization of $10 \pm 1\text{mV}$ (mean \pm S.E., $N=30$) from a resting potential of $-23 \pm 1\text{mV}$. About 80% of the taste cells in the proximal portion of the tongue showed a depolarization of more than 5mV. The taste cells depolarized by deionized water tended to be hyperpolarized by hypertonic NaCl. In contrast, most of the taste cells in the apical region of the tongue were hyperpolarized by deionized water. Return to the normal saline solution led to recovery of the membrane potential to the original level after a further transient depolarization (an off-response).

During stimulation with deionized water, the changes in input resistance of the taste cells ($44 \pm 5\text{M}\Omega$, $N=30$ in the resting state) are classified into three types: a decrease (cell A, $6 \pm 3\text{M}\Omega$, $N=4$), no change (cell B, $N=18$) and an increase (cell C, $6 \pm 2\text{M}\Omega$, $N=8$).

Effect of addition of ions

The gustatory response of the frog glossopharyngeal nerve elicited by deionized water was inhibited by adding a salt to deionized water (Fig. 2A). Adding 0.3mmol l^{-1} NaCl or 0.3mmol l^{-1} choline chloride to deionized water decreased the magnitude of the neural response to about 50% of the control value, whereas 0.3mmol l^{-1} sodium gluconate inhibited the response by only 10% of the control value (Fig. 2B). Similar results have been obtained by Andersson and Zotterman (1950) and Sugawara *et al.* (1989).

The higher the concentration of NaCl added to deionized water, the smaller the amplitude of the water-induced depolarization became. The depolarization was reversed to a hyperpolarization by application of 300mmol l^{-1} NaCl, which was hypertonic compared to the normal saline solution of an adapting solution (Fig. 3A). 100mmol l^{-1} NaBr, 100mmol l^{-1} NaSCN and 200mmol l^{-1} KCl also elicited hyperpolarizing

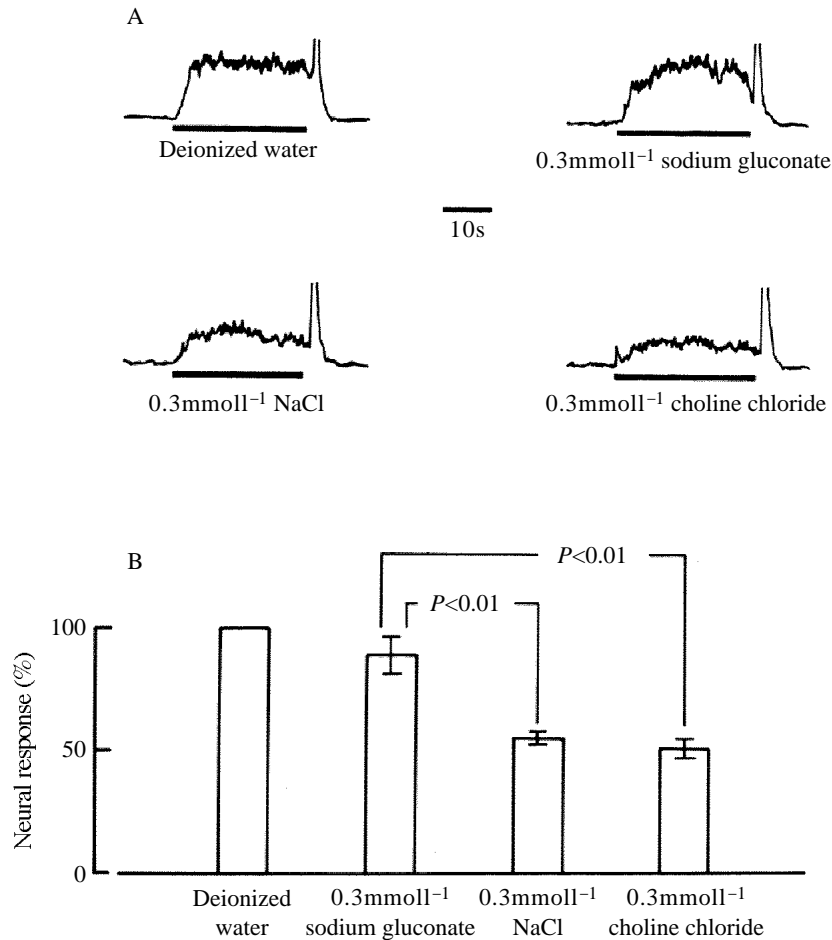


Fig. 2. The integrated frog glossopharyngeal nerve responses elicited by hypotonic salt solutions after adaptation to normal saline. (A) All the records were obtained from the same nerve. (B) The mean amplitudes of the responses expressed as a percentage of the response elicited by deionized water. The column are means of the nerve responses obtained from five nerves and the vertical bars are S.E.M. *P* denotes the level of significance.

responses in water-sensitive taste cells (data not shown). Choline chloride at less than 10mmol l⁻¹ inhibited the water response as strongly as NaCl at the same concentration (data not shown). Low concentrations of sodium gluconate (less than 10mmol l⁻¹) slightly inhibited the depolarization elicited by deionized water, but higher concentration (300mmol l⁻¹) did not cause further inhibition (Fig. 3B).

When 0.1mmol l⁻¹ SITS or 0.1mmol l⁻¹ DIDS, known to block anion channels (Greger, 1990) and anion exchangers (Jennings, 1992), were added to deionized water, the gustatory responses of the taste nerve and cells induced by deionized water were decreased to about 50% of the control value (Fig. 4). However, 0.1mmol l⁻¹ DPC, known to block epithelial anion channels (Greger, 1990), did not significantly inhibit the response (data not shown).

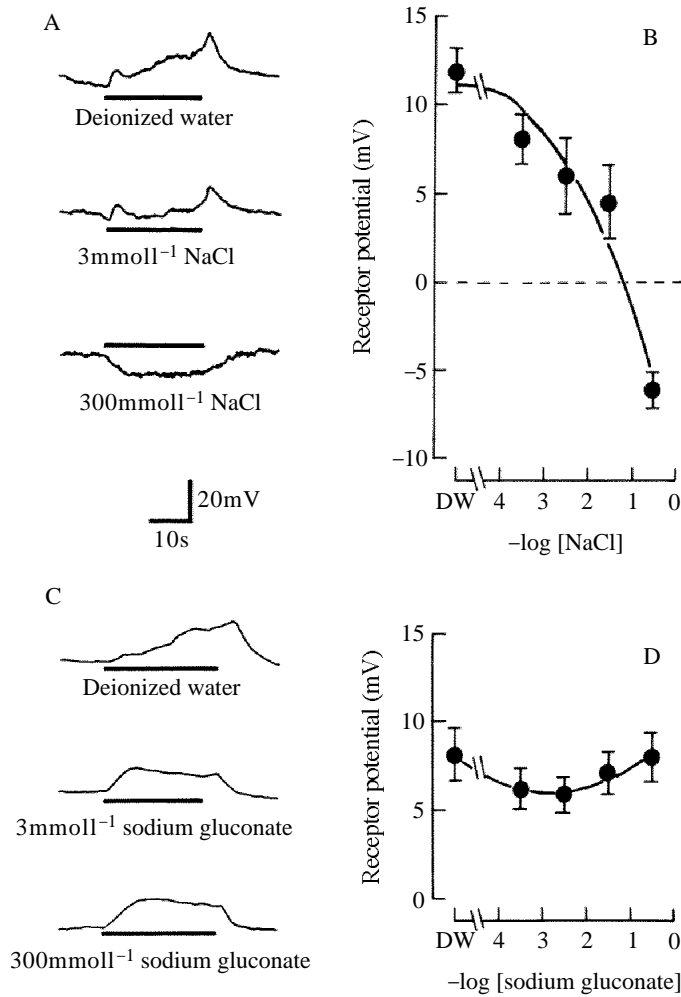


Fig. 3. The receptor potentials elicited by hypotonic and hypertonic salt solutions in frog taste cells after adaptation to normal saline. The receptor potentials were obtained from taste cells whose input resistance was decreased or unchanged by deionized water. (A) All the records were obtained from the same cell. The resting potential was -20mV . (B) The points are means of the receptor potentials obtained from four taste cells and the vertical bars are S.E.M. (C) All the records were obtained from the same cell. The resting potential was -21mV . (D) The points are means of the receptor potentials obtained from six taste cells and the vertical bars are S.E.M. The lines of best fit were drawn by eye.

Reversal potential

Fig. 5 shows two examples of the receptor potentials induced by deionized water in taste cells while their membrane potential was shifted by direct current injection. In taste cells whose input resistance was decreased or unchanged by deionized water, positive shifting of the membrane potential decreased the magnitude of the water response (Fig. 5B). The mean reversal potential of this cell type, calculated by extrapolation, was

$45 \pm 8 \text{ mV}$ (mean \pm S.E., $N=16$). The reversal potential shifted negatively with increasing Cl^- concentration in the stimulating solution (Figs 6B and 7). In contrast, in taste cells whose input resistance was increased during water stimulation, a negative shift of the

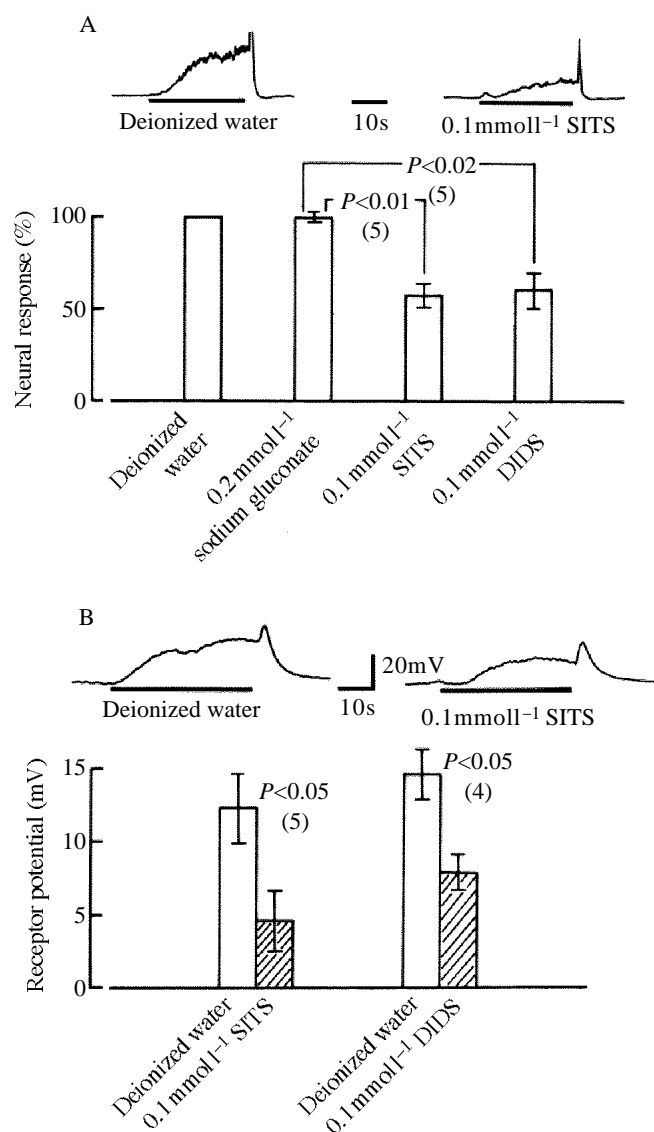


Fig. 4. Effect of addition of SITS or DIDS to deionized water on the taste nerve response (A) and the taste cell response (B). (A) The records are the integrated responses obtained from the same nerve. Amplitudes of the responses are expressed as a percentage of the response elicited by deionized water. (B) Records obtained from the same taste cell. The receptor potential was measured in a taste cell whose input resistance was unchanged by deionized water. The resting potential was -23 mV . The columns are means of the receptor potentials and neural responses and the vertical bars are S.E.M. The numerals in parentheses show the number of taste cells or nerves sampled.

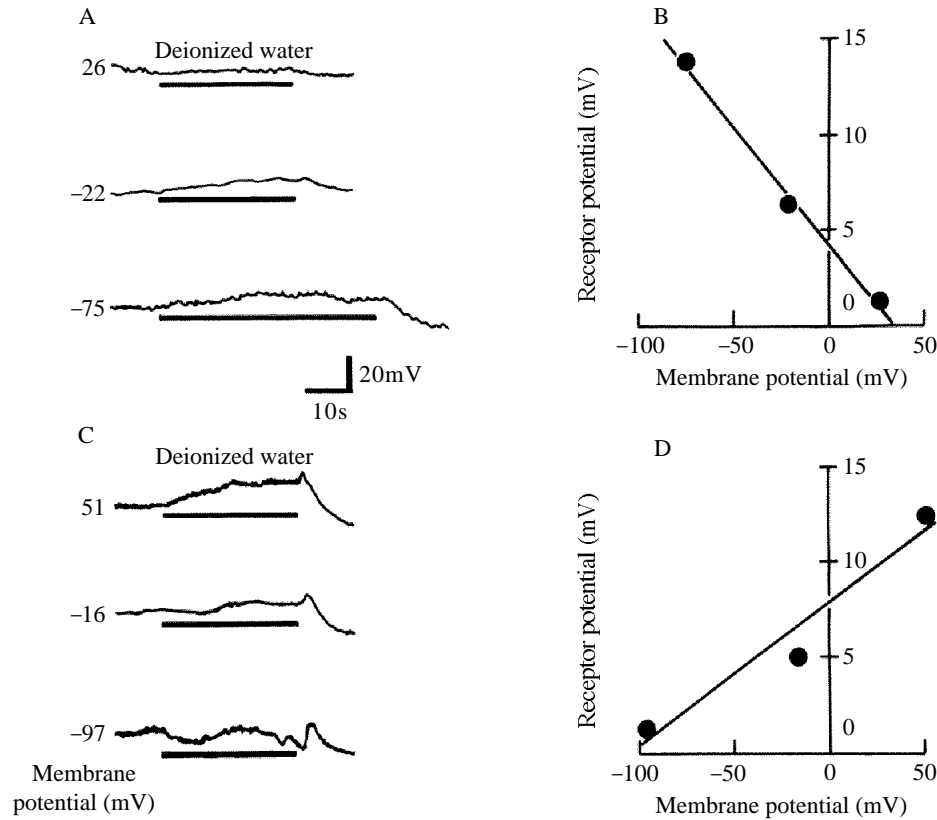


Fig. 5. Relationship between the amplitude of the receptor potential elicited by deionized water and the amplitude of the membrane potential shifted by currents injected into the taste cell. The plots of the responses of the two cells in A and C are given in B and D respectively. The resting potentials were -22mV in A and -16mV in C. A was obtained from a taste cell whose input resistance was unchanged by deionized water and C was obtained from a cell whose input resistance was increased by deionized water. The lines in B and D were fitted using least-squares linear regression.

membrane potential decreased the magnitude of the response (Fig. 5D). The mean reversal potential of this cell type was $-74 \pm 12\text{mV}$ ($N=7$). The reversal potential was independent of Na^+ and Cl^- concentrations in the stimulating solution, but was roughly consistent with the equilibrium potential for K^+ at the basolateral membrane (Fig. 6D) (Sato *et al.* 1984).

The measurement of two groups of reversal potentials suggests that water-sensitive cells can be classified into Cl^- -dependent cells (about 70%), whose input resistance was decreased or unchanged by deionized water, and Cl^- -independent cells (about 30%), whose input resistance was increased by deionized water.

Injection of Cl^- into a taste cell

Fig. 8 shows the effect of injecting Cl^- into a taste cell on the receptor potential elicited by deionized water. When Cl^- was injected into a cell whose input resistance was

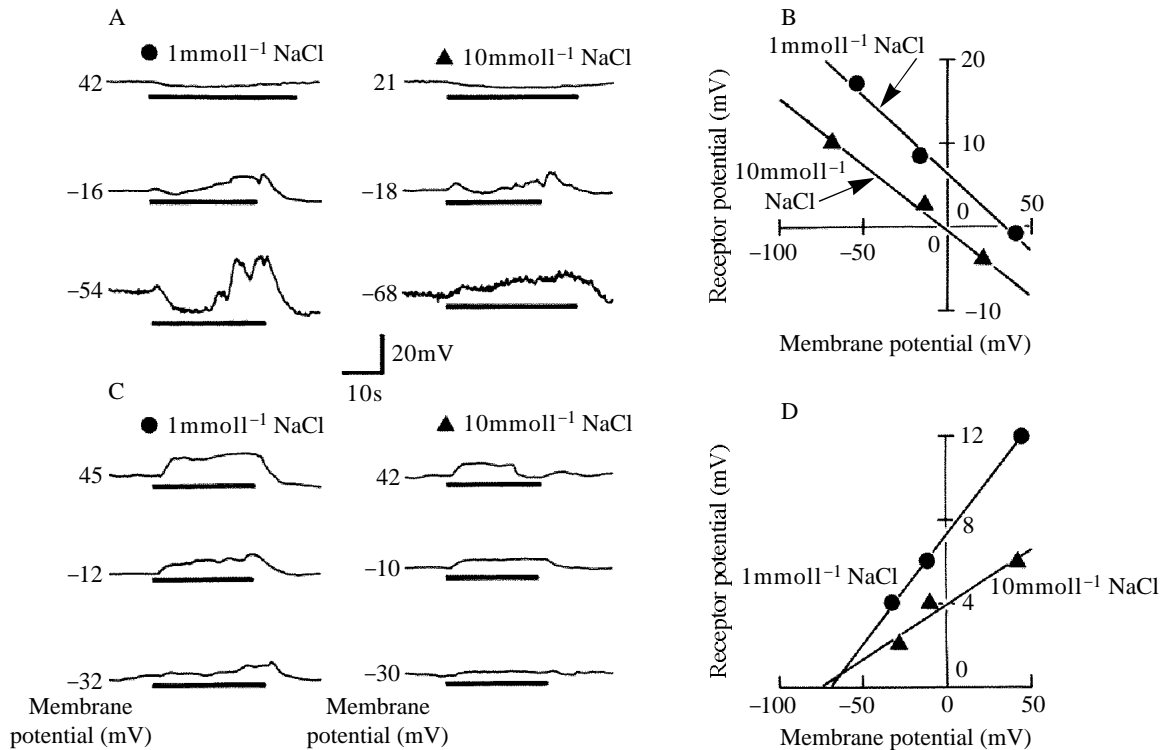


Fig. 6. Relationship between the amplitude of the receptor potential elicited by hypotonic NaCl solutions and the amplitude of the membrane potential. The plots of the responses of the two cells in A and C are given in B and D respectively. The resting potentials were -18 mV in A and -30 mV in C. A was obtained from a taste cell whose input resistance was unchanged by deionized water and C was obtained from a cell whose input resistance was increased by deionized water. The lines in B and D were fitted using least-squares linear regression.

unchanged by deionized water, the magnitude of the receptor potential elicited by a water stimulus increased (Fig. 8A). When Cl^- was injected into a cell whose input resistance was increased by deionized water, the receptor potential did not change (Fig. 8B).

Effects of interstitial ions

Fig. 9 shows the effect of exchanging the normal interstitial fluid with modified saline solutions on the resting potential and water-induced depolarization in frog taste cells. When interstitial Na^+ was replaced with choline, the amplitude of the resting potential increased to 140% of the control value and the depolarizing receptor potential was reversed to a hyperpolarization. Exchange of Cl^- with gluconate also increased the resting potential to 135% of the control value and reversed the polarity of the receptor potential. When interstitial Na^+ and Cl^- were replaced by sucrose, the resting potential increased to 170% of the control value and the amplitude of the hyperpolarizing receptor potential was reversed to the same extent as the amplitude in either Na^+ -free or Cl^- -free interstitial fluid. All of these inhibitory effects were reversible. The use of K^+ -free interstitial fluid did not affect the water response (data not shown).

When 1mmol l^{-1} furosemide, known to inhibit Na^+/Cl^- cotransport (O'Grady *et al.* 1990) and anion exchange (Jennings, 1992), was added to the interstitial fluid, the resting potential increased to 125% of the control value and the receptor potential decreased reversibly to 20% of the control value. Neither interstitial 0.1mmol l^{-1} Na_3VO_4 (an

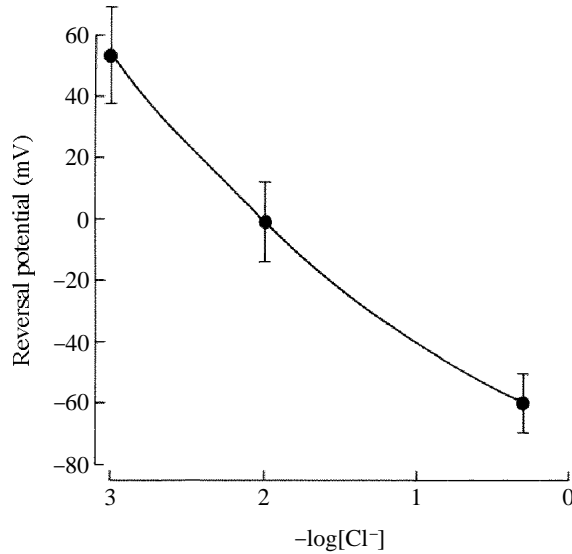


Fig. 7. Relationship between the Cl^- concentration of the stimulating solution and the reversal potential of the receptor potential. The reversal potentials were obtained from taste cells whose input resistance was decreased or unchanged by deionized water. The points are means of the reversal potentials obtained from 4–9 cells and the vertical bars are S.E.M. The curve was fitted by the least-squares method.

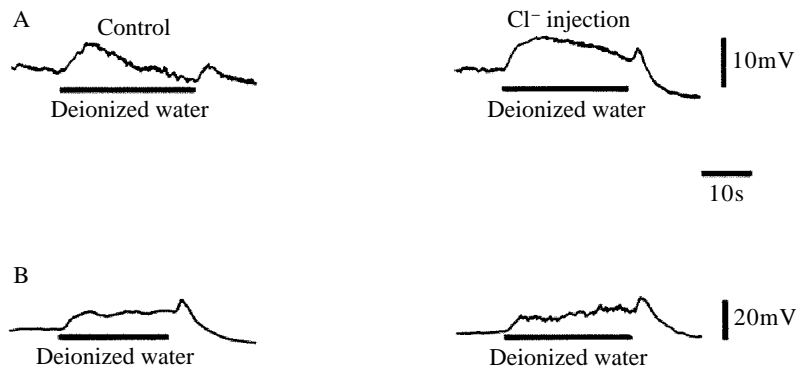


Fig. 8. Effect of injecting Cl^- into a taste cell on the receptor potential elicited by deionized water. A was obtained from a taste cell whose input resistance was unchanged by deionized water and B was obtained from a cell whose input resistance was increased by deionized water. The reversal potential of the receptor potential in A was 35mV and the resting potential was changed from -25 to -22mV during Cl^- injection. The reversal potential in record B was -48mV and the resting potential was shifted from -19 to -25mV during Cl^- injection.

inhibitor of ATPases) nor 1mmol l^{-1} Cd^{2+} (a blocker of Ca^{2+} channels) affected the water-induced receptor potential (data not shown).

When 2mmol l^{-1} Ba^{2+} , known to block K^{+} channels, was added to the interstitial fluid, the glossopharyngeal nerve response induced by deionized water decreased reversibly to 60% ($N=4$) of the control value (Fig. 10). Since the exchange of normal interstitial fluid

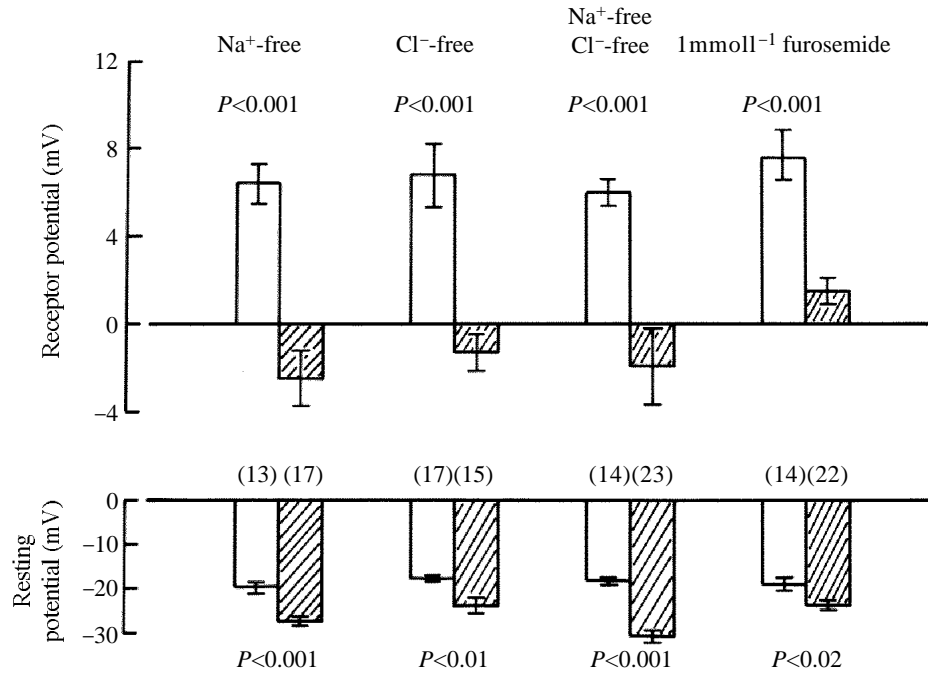


Fig. 9. Effects of changing interstitial ion compositions and adding furosemide on the resting potentials and the receptor potentials induced by deionized water. The open columns are the control responses and the hatched columns are the test responses. The numerals within parentheses show the number of taste cells sampled and the vertical bars are S.E.M. P denotes the level of significance.

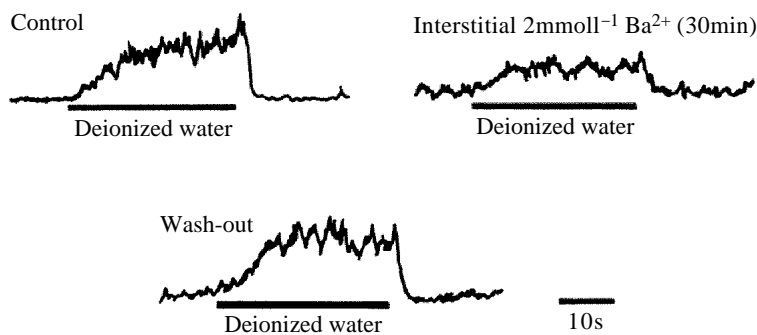


Fig. 10. Effect of interstitial 2mmol l^{-1} Ba^{2+} on the integrated response of the frog glossopharyngeal nerve elicited by deionized water. The records were obtained from the same nerve.

with Ba^{2+} -containing fluid induced oscillatory contractions of the tongue muscle, it was impossible to insert a microelectrode into a taste cell in the presence of interstitial Ba^{2+} . Therefore, we could not examine the effect of interstitial Ba^{2+} on water-induced receptor potentials.

Discussion

In frog taste cells whose input resistance was decreased or unchanged by deionized water, receptor potentials were inhibited by adding chloride salts or Cl^- channel blockers. The reversal potentials for the receptor potentials shifted in the hyperpolarizing direction as the Cl^- concentration in the water stimulus was increased (Figs 3, 4, 6). Furthermore, the magnitude of the receptor potential was enhanced by injecting Cl^- into a taste cell (Fig. 8). About 70% of the taste cells sensitive to deionized water were Cl^- -dependent cells. It is suggested that Cl^- channels may exist in the taste-receptive membrane in these cells. Upon water stimulation, Cl^- may be secreted from the receptive membrane of the taste cell by the driving force through Cl^- channels which were open before water stimulation or are activated after the stimulation. This leads to a depolarization in the Cl^- -dependent taste cells. The mean reversal potential ($45 \pm 8 \text{ mV}$) for the water response of Cl^- -dependent cells was approximately equivalent to the reversal potential ($54 \pm 15 \text{ mV}$) for the response elicited by 1 mmol l^{-1} NaCl. This suggests that the local equilibrium concentration of Cl^- in the vicinity of the receptive site during water stimulus is about 1 mmol l^{-1} .

Transepithelial resistance can be affected by either the transcellular or paracellular pathways. The paracellular pathways under the present experimental conditions are considered to reside in the tight junctions of the lingual epithelium and in the steel pins used for fixing the tongue. Since the transepithelial resistance of the bullfrog is as high as $710 \text{ } \Omega \text{ cm}^2$ under symmetrical ionic conditions (Soeda and Sakudo, 1985), it is likely that the major paracellular pathway, having negligible low resistance, is formed by the steel pins. This may account for the observation that the apical membrane potential was equal to the basolateral membrane potential. It is not clear why some Cl^- -dependent cells displayed a decrease in input resistance during stimulation by deionized water. This stimulus might activate swelling-sensitive Cl^- -channels which were closed under resting conditions. Similarly, the apical resistance changes of *Necturus* taste cells could be measured by the indifferent electrode with reference to the interstitial fluid, although the transepithelial resistance was very large (about $2000 \text{ } \Omega \text{ cm}^2$) (Roper and McBride, 1989). In these experiments, the input resistance was measured by intracellular current injection using a bridge circuit. The resistance determined by this method might consist of apical and basolateral components of the membrane resistance. The basolateral membrane resistance can be measured accurately by transepithelial current injection while measuring intracellular membrane potential with reference to the interstitial side.

Elliott and Simon (1990) reported that the response of rat chorda tympani nerve to NaCl was not modified by 0.1 mmol l^{-1} SITS. They postulated that the anion effect on salt taste is mediated by the paracellular pathway. In the dog, whose gustatory nerve displays impulse discharges in response to water (Liljestrand and Zotterman, 1954), the

lingual epithelium actively secretes Cl^- (Mierson *et al.* 1985). In the frog, SITS (1mmol l^{-1}) enhanced the glossopharyngeal nerve response elicited by NaCl and shifted positively the reversal potential of the NaCl-induced receptor potential in the taste cell (Y. Okada, unpublished data). Furthermore, SITS (0.1mmol l^{-1}) inhibited the water response (Fig. 4). These results suggest that the anion-sensitivity of frog and dog taste cells may be different from that of the rat, and that frog and dog taste cells may use transcellular Cl^- transport.

Taste cells that were depolarized by water stimulation displayed a hyperpolarization in response to hypertonic NaCl solution. However, at high superficial Cl^- levels, the reversal potential was more positive than the equilibrium potential for Cl^- , assuming that the intracellular Cl^- concentration was 10mmol l^{-1} (Fig. 7). The existence of Na^+ permeability in the gustatory receptive membrane (Okada *et al.* 1986; Miyamoto *et al.* 1989) or a positive shift in the equilibrium potential for Cl^- due to Cl^- entry during NaCl stimulation may account for this deviation from Cl^- equilibrium.

When water is perfused onto the surface of the tongue, the receptive membrane of taste cells is exposed to a hypotonic solution. A hypotonic solution may cause cell swelling. Studies using the patch-clamp technique have shown that some epithelial cells have swelling-activated Cl^- channels (Hazama and Okada, 1988; McCann *et al.* 1989; Solc and Wine, 1991). It was suggested that swelling-sensitive Cl^- channels might be modulated by arachidonic acid metabolites (Doroshenko and Neher, 1992). Water-induced Cl^- secretion in frog taste cells may occur through swelling-sensitive Cl^- channels. Further investigation of the water response using the patch-clamp technique is necessary.

The reversal potentials for receptor potentials in the other type of taste cells, which showed an increased input resistance in response to stimulation with deionized water, were not influenced by superficial Cl^- concentration, but were roughly consistent with the equilibrium potential for K^+ at the basolateral membrane (Fig. 6D). The equilibrium potential for K^+ with reference to the interstitial fluid in frog taste cells has been calculated to be about -100mV according to the constant field theory (Sato *et al.* 1984). Recent patch-clamp experiments showed that application of cyclic AMP with ATP to a frog taste cell depolarized the membrane potential and inhibited the voltage-activated K^+ currents (Avenet and Lindemann, 1987; Avenet *et al.* 1988; Okada *et al.* 1990). Water stimulation may depolarize about 30% (the Cl^- -independent population) of the water-sensitive taste cells by inhibiting the resting K^+ conductance *via* cyclic-AMP-dependent phosphorylation. Inhibition of the water-induced response in the glossopharyngeal nerves by interstitial Ba^{2+} suggests that the K^+ conductance mediating the water response is located in the basolateral membrane of the taste cell (Fig. 10). The responses elicited by NaCl, HCl, quinine and galactose in those nerves were not inhibited by interstitial Ba^{2+} (Y. Okada, unpublished data). However, there is no evidence that water stimuli (low osmolarity) elevate the cyclic AMP concentration inside the taste cell and it is not clear whether a second messenger produced at the receptive membrane can reach the basolateral membrane. Interstitial Ba^{2+} also inhibited the short-circuit current in the rat lingual epithelium *in vitro* with hyperosmotic KCl on the luminal side (Simon *et al.* 1988). This effect of Ba^{2+} in rat suggests that the short-circuit current may be composed

of apical K^+ entry and serosal K^+ efflux, and that interstitial Ba^{2+} may inhibit serosal K^+ efflux. In other words, since the short-circuit current is composed of apical depolarizing and serosal hyperpolarizing pathways, it is very difficult to estimate the change in the intracellular membrane potential of taste cells from the polarity of the current. However, frog and rat taste cells can use the same mechanism in the taste response to KCl (Miyamoto *et al.* 1989) and these cells may have Ba^{2+} -sensitive K^+ channels in the basolateral membrane.

Adding salts to deionized water decreased the magnitude of the receptor potential in Cl^- -independent water-sensitive taste cells (Fig. 6D). Therefore, not all the inhibitory effects of added salts on the water response can be attributed to a reduction in the driving force for Cl^- secretion through the receptive membrane. There is evidence in cat fungiform papillae that the osmolarity in the papillae changes with different stimuli (Hallbäck *et al.* 1979). Changes in interstitial osmolarity can be effected by either transcellular or paracellular pathways (Fidelman and Mierson, 1989). The paracellular pathway is known to play a role in gustation (DeSimone *et al.* 1984; Harper, 1987; Ye *et al.* 1991). The inhibitory effects of added salt on Cl^- -independent cells may be caused by a change in interstitial osmolarity through the paracellular pathway.

Cl^- secretion through the taste-receptive membrane in response to stimulation with water suggests that the frog taste cell is capable of accumulating Cl^- inside the cell. There is a furosemide-sensitive Na^+/Cl^- cotransporter in the basolateral membrane of the taste cell (Okada *et al.* 1988c). Na^+/Cl^- cotransport can build up the driving force for Cl^- secretion. The elimination of interstitial Cl^- in the apical and middle regions of frog tongue did not affect the resting potential of the taste cells (Sato *et al.* 1984; Okada *et al.* 1986). In the present experiments with taste cells in the proximal region of the tongue, interstitial Cl^- -free and furosemide-containing fluids shifted the resting potentials in the hyperpolarizing direction (Fig. 9). This suggests that the taste cells that secrete intracellular Cl^- in response to water stimulation may have a resting potential dependent on Cl^- as well as on K^+ and Na^+ .

The use of Na^+ -free and Cl^- -free interstitial fluids reversed the water-induced depolarization to a hyperpolarization (Fig. 9). Even if intracellular Cl^- concentration is decreased by the inhibition of Na^+/Cl^- cotransport, water stimulation without Cl^- will not induce a hyperpolarization based on the Cl^- permeability. It is possible that the reduction in interstitial osmolarity through the paracellular pathway during water stimulation may induce the hyperpolarization of taste cells after elimination of the furosemide-sensitive transcellular pathway for Cl^- secretion. This suggests that the paracellular pathway during water stimulation may affect taste cells negatively even when the transcellular pathway for Cl^- is active.

In conclusion, water-induced depolarizing receptor potentials are attributed to Cl^- secretion through the taste-receptive membrane in about 70% of frog taste cells and to the inhibition of the resting K^+ conductance of the basolateral membrane in the remaining 30% of the cells.

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