

Membrane Resistance Change of the Frog Taste Cells in Response to Water and NaCl

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ABSTRACT The electrical properties of the frog taste cells during gustatory stimulations with distilled water and varying concentrations of NaCl were studied with intracellular microelectrodes. Under the Ringer adaptation of the tongue, two types of taste cells were distinguished by the gustatory stimuli. One type, termed NaCl-sensitive (NS) cells, responded to water with hyperpolarizations and responded to concentrated NaCl with depolarizations. In contrast, the other type of cells, termed water-sensitive (WS) cells, responded to water with depolarizations and responded to concentrated NaCl with hyperpolarizations. The membrane resistance of both taste cell types increased during the hyperpolarizing receptor potentials and decreased during the depolarizing receptor potentials. Reversal potentials for the depolarizing and hyperpolarizing responses in each cell type were a few millivolts positive above the zero membrane potential. When the tongue was adapted with Na-free Ringer solution for 30 min, the amplitude of the depolarizing responses in the NS cells reduced to 50% of the control value under normal Ringer adaptation. On the basis of the present results, it is concluded (*a*) that the depolarizing responses of the NS and WS cells under the Ringer adaptation are produced by permeability increase in some ions, mainly Na⁺ ions across the taste cell membranes, and (*b*) that the hyperpolarizing responses of both types of taste cells are produced by a decrease in the cell membrane permeability to some ions, probably Na⁺ ions, which is slightly enhanced during the Ringer adaptation.

INTRODUCTION

Concerning transduction of gustatory stimuli into the electrical responses of taste cells, the following three successive events have been proposed (Beidler, 1954, 1961 *a*, *b*, 1967, 1969, 1971; Beidler and Gross, 1971): (*a*) weak adsorption of stimulus molecules or ions to the macromolecules of exposed surface receptor membranes of taste cells, (*b*) conformational changes in the receptor macromolecules themselves, which trigger structural changes in the taste cell membranes distant from the receptor membranes of the micro-

villi, and (c) change in ionic permeability of the cell membranes which results in the generation of receptor potentials. Since the biochemical research by Dastoli and Price (1966), taste receptor macromolecules which form complexes with gustatory stimuli have been obtained from the gustatory epithelia (Hiji et al., 1971; Kurihara et al., 1972; Lo, 1973). This finding seems to support Beidler's hypothesis (1954) as to the first event of the transduction process described above, though the question remains whether the receptor molecules obtained originate from the taste receptor membrane proper. No experimental study has been reported on the conformational change in the taste receptor macromolecules nor the taste cell membranes. Kimura and Beidler (1961) first recorded depolarizing receptor potentials from the rat and hamster taste cells with intracellular microelectrodes. They found that single taste cells responded to multiple gustatory stimuli by producing different amplitudes of receptor potentials. This finding was subsequently confirmed by Sato (1969, 1972) and Akaike et al. (1973) in frogs and by Tateda and Beidler (1964) and Ozeki and Sato (1972) in rats. Recently Ozeki (1970, 1971) has examined the membrane conductance change of rat taste cells in response to basic taste stimuli and has found that the conductances increase during the depolarizations produced by stimuli NaCl, sucrose, and HCl, and decrease during the depolarizations produced by quinine-HCl.

It is well known that the frog gustatory receptors respond to water with conspicuous neural discharges (Zotterman, 1949; Andersson and Zotterman, 1950). Frog water receptors are known to be highly sensitive to calcium ions (e.g., Nomura and Ishizaki, 1972). On the other hand, it is generally accepted that little water response is found in the rat's taste receptors. Therefore, frog gustatory receptors are advantageous material for studying the properties of the receptor potentials which are responsible for the water responses in the gustatory nerves.

In the present experiments we undertook to examine the relationships between the receptor potentials and the changes in membrane resistance of the frog taste cells during gustatory stimulation with water and various concentrations of NaCl. In addition, in order to understand the ionic mechanisms concerning the generation of receptor potentials, we examined reversal potentials for the receptor potentials and the effects of Na-free Ringer and water adaptations on the taste cell responses. Preliminary short communications have appeared (Sato and Greenberg, 1972; Sato and Beidler, 1973).

MATERIALS AND METHODS

Preparation

The experiments were performed on the tongues of 70 leopard frogs (*Rana pipiens*) and 20 bullfrogs (*Rana catesbeiana*) without any appreciable difference in experimental results. All frogs contributed to data presented in the Results. In most experiments

the animals were deeply anesthetized with urethane (2.0 ml/kg body weight), but in some experiments the central nervous system was pithed in order to immobilize the animals. The hypoglossal nerves of both sides were severed. The tongue was pulled out from the mouth and was fixed with several insect pins on the cork plate of an experimental chamber. In addition to the *in situ* experiments, isolated tongues were employed in several experiments.

Gustatory Stimulation

Distilled water (conductivity $<10^{-7}$ mho/cm) and various NaCl concentrations of 10^{-5} –1.0 M made up in distilled water were used as gustatory solutions. These stimulus solutions were applied to the tongues by modifying the method of Suzuki and Tucker (1971). The frog Ringer solution, which was stocked in a reservoir of 500-ml glass bottles, was flowed continuously over the tongue surface as an adaptation medium using polyethylene tubing of 0.5-mm internal diameter connected to the bottle. The Ringer saline used had the following composition: NaCl, 111.2 mM; KCl, 1.9 mM; CaCl_2 , 1.1 mM; NaH_2PO_4 , 0.1 mM; and NaHCO_3 , 2.4 mM (a pH of 7.2–7.5). All chemicals were reagent grade. A small glass tube with an injection port was provided in a part of the tubing for the introduction of gustatory stimuli. The position of both the Ringer reservoir and the injection tube was adequately adjusted for making a very thin layer of the Ringer flow at the bottom of the injection tube. Thus, a flow of 1.4 ml/min of Ringer's resulted. When stimulating the tongue, gustatory solutions of 0.3–1.0 ml were injected into the injection port. Immediately after the injection, the Ringer flow was stopped by pressing the tubing of the Ringer reservoir side with a valve. After the injected stimulus solutions disappeared, the valve was released to restart the Ringer flow. In this procedure, mixing of the stimulus solution with the Ringer occurred. The time-course of this mixing was determined by injecting methylene blue solution into the injection tube and by measuring the change of the concentrations at the nozzle located on the tongue by means of a spectrophotometer. It was estimated that the stimulus concentration at the nozzle reached 96% of the injected concentration within 10.4 s, and Ringer replacement occurred within 15.6 s.

When recording microelectrodes were inserted into a fungiform papilla located just beneath the nozzle, the microelectrodes were occasionally withdrawn by mechanical vibration from the solution flow. Therefore, the microelectrodes were usually inserted into the papillae 10 mm or less from the nozzle. Since the tongue was covered with a thin layer of both the flowing Ringer and the mucus, the peak and fall times of stimulus concentrations over the inserted papillae lengthened further as a function of a distance between the microelectrode tip and the nozzle on the tongue. The time-course of taste receptor potentials varied as a function of this distance. The approximate period of stimulus application was marked on the pen recorder as the time interval between onset of the stimulus injection and onset of the Ringer flow into the injection tube.

Recording

Glass capillary microelectrodes were used to record intracellular receptor potentials from the taste cells of the fungiform papillae. The electrodes were filled with 2 M

KCl and had a DC resistance of 20–60 M Ω in Ringer saline. A reference electrode of thick Ag-AgCl wire was inserted into the musculature of the forelimb. In the case of isolated tongue preparations, a reference electrode of Ringer-filled glass pipette of about 10- μ m diameter was inserted into the tongue muscles.

Microelectrode potentials were fed into a high input impedance DC preamplifier (Grass P16, Grass Instrument Co., Quincy, Mass.) via a thin chlorinated silver wire. The amplified electrical responses were monitored on the oscilloscope (Tektronix 502A, Tektronix, Inc., Beaverton, Ore.) and recorded with a pen recorder (frequency characteristic, 0–100 Hz). A jolting device (Nihon Kohden MID-1, Nihon Kohden Kogyo Co., Tokyo, Japan; see Tomita et al., 1967) was used to facilitate the penetration of cells. A small experimental tongue chamber was placed on the jolter diaphragm, which was driven at a frequency of 0.5 Hz with a pulse generator.

The membrane potential of taste cells was changed by passing depolarizing or hyperpolarizing currents through a recording microelectrode connected to a Wheatstone bridge. The membrane resistance of taste cells before, during, and after gustatory stimulations was determined by applying hyperpolarizing constant current pulses of 100–200-ms duration at a frequency of about 0.6 Hz and by measuring voltage drop across the cell membrane. Applied current across the membrane was amplified with another DC preamplifier (Grass P6), monitored on the oscilloscope, and recorded with the pen recorder.

Procedure of Penetration of Cells

The taste cells of the frog tongue are found only in the fungiform papillae. The structure of the frog taste organ (taste disk) is different from that of a typical mammalian taste organ (taste bud). The frog taste disk covers almost the entire summit of the fungiform papilla and contains cell bodies of supporting cells in the upper layer and those of taste cells in the lower layer (DeHan and Graziadei, 1971; Graziadei and DeHan, 1971). Although taste cells extending the dendrite-like processes are located across the entire length of the taste disk, it seems possible for the microelectrode to penetrate only the taste cell bodies in the two inferior thirds of the taste disk. This cell body layer originates at 15- μ m depth from the papilla surface with 25- μ m thickness. Thus, the microelectrode tip was vertically advanced rapidly within the cell body layer by means of a micromanipulator (Narishige MZ-10, Narishige Scientific Instrument Laboratory, Tokyo, Japan) and the penetration was aided with the jolting action. The penetration of cells was signaled by a sudden appearance of both negative resting potential and effective membrane resistance which could be measured with the aid of a Wheatstone bridge. In most cases the resting potential recorded reduced gradually to the depolarizing direction: this was probably due mainly to damage of the membranes of small cells of 6–10- μ m diameter. When the resting potentials were stable for 1 min or more after the penetration, gustatory stimuli were given to the cells.

Microelectrodes were assumed to penetrate the taste cells according to the following criteria: (a) generation of the slow potentials, receptor potentials, of either polarity in response to stimuli, (b) change of the cell membrane resistance during stimuli, (c) return of the slow potentials to the original resting potential after the Ringer

rinse, (d) return of the changed resistance to that at rest after the rinse, and (e) return of the resting potential to the zero membrane potential after withdrawal of the electrode.

Identification of Recording Sites

Since the cytoplasmic processes of the superficially located supporting cells in the frog taste disk extend down to the taste cell bodies, there is a possibility that a microelectrode tip penetrates these processes even if it is advanced into the taste cell body layer. In addition one would like to discriminate between slow potentials as taste receptor potentials and artifacts produced between microelectrode tips and gustatory solutions. Therefore, it is of importance to demonstrate that a microelectrode is truly inside the taste cell and that the recorded slow potentials are physiological. One of the best methods to clarify this is to stain the cell electrophoretically with dye-filled microelectrodes after recording of the gustatory responses. Glass capillary microelectrodes were filled with the following dyes dissolved in distilled water: (a) 4–5% Niagara Sky Blue 6B, (b) 5% Procion Yellow M-4RS, and (c) a mixture of 3% Niagara Sky Blue and 3% Procion Yellow. The capillaries were filled with Niagara Sky Blue using a boiling method (Kaneko and Hashimoto, 1967), and with Procion Yellow and the mixture of dyes using the method of Tasaki et al. (1968). These dye-filled microelectrodes had a DC resistance of 30–80 M Ω . The method of iontophoretic injection of dyes into cells was similar to that used by Kaneko and Hashimoto (1967), Kaneko (1970), and Ozeki (1973). Niagara Sky Blue was injected with a negative current of about 10^{-6} A applied to the microelectrodes for 2–20 s, while Procion Yellow and the mixed dyes were injected with a negative current of 10^{-7} – 10^{-8} A for 5–60 s. The amount of current was always monitored on the oscilloscope (Tektronix 502A) during the dye injections. Current intensity and injection time were varied according to microelectrode resistance and polarization. With Niagara Sky Blue and the mixed dyes under a dissecting binocular microscope it was possible to observe an injected dye spot within the fungiform papilla. However, no Procion Yellow dye spot could be observed under an ordinary light microscope, so the injection of this dye was achieved only by the current monitor. After dye injection, the tongue was undisturbed for more than 30 min and then the tongue was fixed with 4% glutaraldehyde solution adjusted to a pH 4 with acetate buffer, dehydrated with methanol, embedded into paraffin, and cut into 10- μ m serial sections. All the experiments were performed at room temperature ranging from 25 to 27°C.

RESULTS

General Properties of Taste Cell Responses

When the tongue was adapted with the Ringer saline, taste cells were distinguished into two types from the intracellular responses to distilled water and to varying concentrations of NaCl: One type produced hyperpolarizing responses to applications of water and low concentrations of NaCl and pro-

duced depolarizing responses to high concentrations of NaCl (Fig. 1 A). This type is here termed NaCl-sensitive cells (NS cells). On the contrary, the other type of taste cells responded to water and low concentrations of NaCl by producing depolarizing responses and responded to high concentrations of NaCl by producing hyperpolarizing responses (Fig. 1 B). This type is termed water-sensitive cells (WS cells). Spike potentials were recorded from neither the NS nor the WS cells. The time-course of depolarization and hyperpolarization

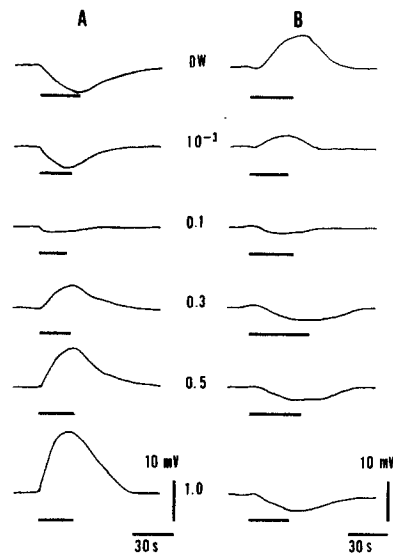


FIGURE 1. Intracellular recordings of receptor potentials of single taste cells in response to distilled water and NaCl of varying concentrations, (A) from a NS cell, (B) from a WS cell. In records A and B, upward deflections denote depolarizations and downward deflections, hyperpolarizations. An abbreviation and numerals shown between records A and B indicate gustatory stimuli of distilled water (DW) and NaCl of various molar concentrations. In this and subsequent figures, horizontal bars shown beneath each record indicate the period of stimulus applications. Resting potentials were about -14 mV in A and -22 mV in B.

zation responses in both NS and WS cells was slow: the peak time after stimulus application was 15–40 s and the fall time after rinsing 25–70 s. The rate of rise of depolarizations and hyperpolarizations increased with increasing taste stimulus concentrations (Figs. 1, 11).

The resting potentials of the NS cells, which were measured shortly after the penetration of cells, were -8.0 to -47.0 mV with an average value of -17.8 ± 0.6 mV (SE of mean, $n = 198$), the resting potentials of the WS cells being -7.8 to -41.0 mV with an average of -19.2 ± 1.0 mV (SE, $n = 53$). No significant difference was found between the resting potentials in both types ($P > 0.1$, t test). These values of the resting potentials were

slightly lower than those of previous measurements (Sato, 1972). This is probably due to lower resistances of the microelectrodes employed in the present work.

The input resistances of the NS cells were 10–182 M Ω with mean 39.6 ± 4.1 M Ω (SE, $n = 104$), those of the WS cells being 10–99 M Ω with mean 32.0 ± 4.6 M Ω (SE, $n = 31$). These values in both cell types were not significantly different ($P > 0.1$, t test). The relation of the membrane potential changes (V) to passage of depolarizing or hyperpolarizing currents (I) across the taste cell membrane was measured using a Wheatstone bridge. The I - V relations in both cells were found to be linear within about ± 60 -mV displacement in the membrane potentials. Fig. 2 shows an example of I - V

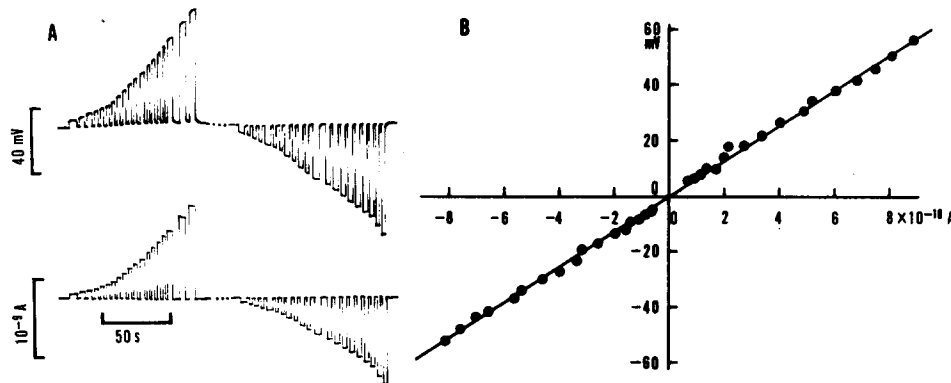


FIGURE 2. Relation between applied currents (I) across a NS-cell membrane and electrotonic potentials (V) intracellularly recorded from the same cell. In A, upper trace indicates electrotonic membrane potentials and lower trace applied currents. In upper and lower traces, upward deflections denote depolarizing potentials and depolarizing currents, respectively. (B) I - V relation obtained from A. This NS cell had an input resistance of 62 M Ω which was calculated from a slope of straight line showing I - V relation.

relation in a NS cell. Fig. 2 A denotes the amplitudes of membrane potential changes (upper trace) and the intensities of applied currents (lower trace), and Fig. 2 B a plotting of these relations. This NS cell had an input resistance of 63 M Ω which was calculated from the straight line through the plotted points.

The distribution ratio of the NS and WS cells was examined both in the anterior half and in the posterior half of the tongue. The definition of anterior and posterior parts is the same as that of the mammalian tongue, though the frog is capable of projecting the whole tongue beyond the jaws (Noble, 1954). That is, the anterior half of the tongue is defined as the half area of the jaw side and the posterior half as the remaining half area of the opposite side. It was calculated that the anterior part of the tongues had 48% NS

cells ($n = 48$ in eight preparations) and 52% WS cells ($n = 52$ in the same preparations), whereas the posterior part had 80% NS cells ($n = 16$ in three preparations) and 20% WS cells ($n = 4$ in the same preparations)

Relation between Stimulus Concentrations and Receptor Potentials

Fig. 3 shows the relationships between the stimulus concentrations of NaCl and the amplitudes of receptor potentials in the NS cells (A) and the WS cells (B). In Fig. 3 A the responses of four different NS cells are plotted.

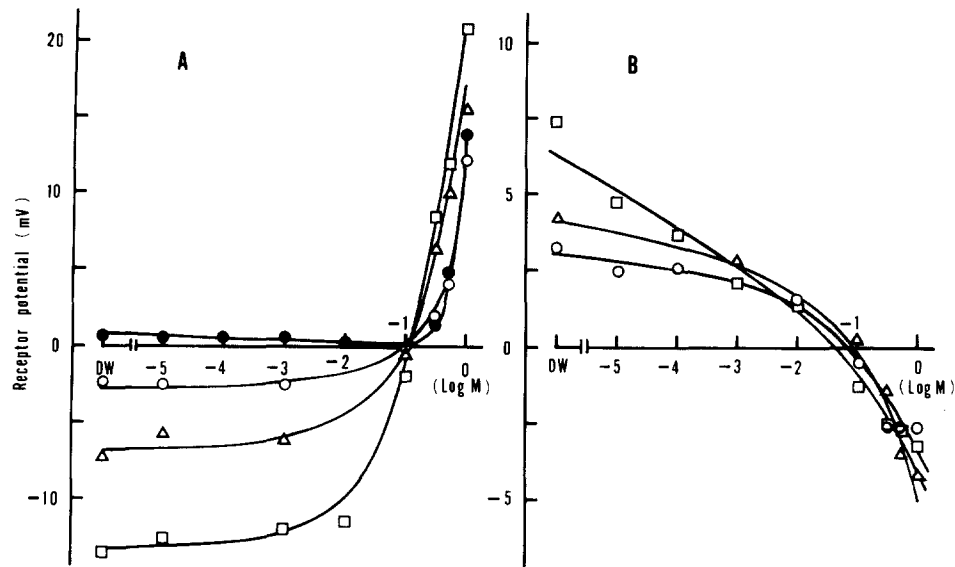


FIGURE 3. Relations between stimulus concentrations and peak amplitudes of receptor potentials, (A) from four different NS cells, (B) from three different WS cells. In abscissae, NaCl concentrations are shown in logarithm of molar concentrations and DW denotes distilled water. In ordinates, positive numerals denote depolarizing potentials. Resting potentials of the NS cells (A) shown by closed circles, open circles, triangles, and squares were, respectively, around -26 , -30 , -15 , and -19 mV, and resting potentials of the WS cells (B) shown by squares, triangles, and circles were, respectively, around -20 , -20 , and -18 mV.

Out of the four cells, the three are typical NS cells which produced the hyperpolarizing responses to water and NaCl concentrations less than 0.1 M and produced the depolarizing responses to NaCl greater than 0.1 M. The amplitudes of depolarizations increased greatly as NaCl concentrations were increased above 0.1 M. On the other hand, the amplitudes of hyperpolarizations increased slowly as NaCl concentrations were decreased below 0.1 M. In one cell plotted with closed circles in Fig. 3 A, depolarizing responses were observed over all ranges of NaCl concentrations and water. In this cell the depolarizations elicited by water and low NaCl concentrations were

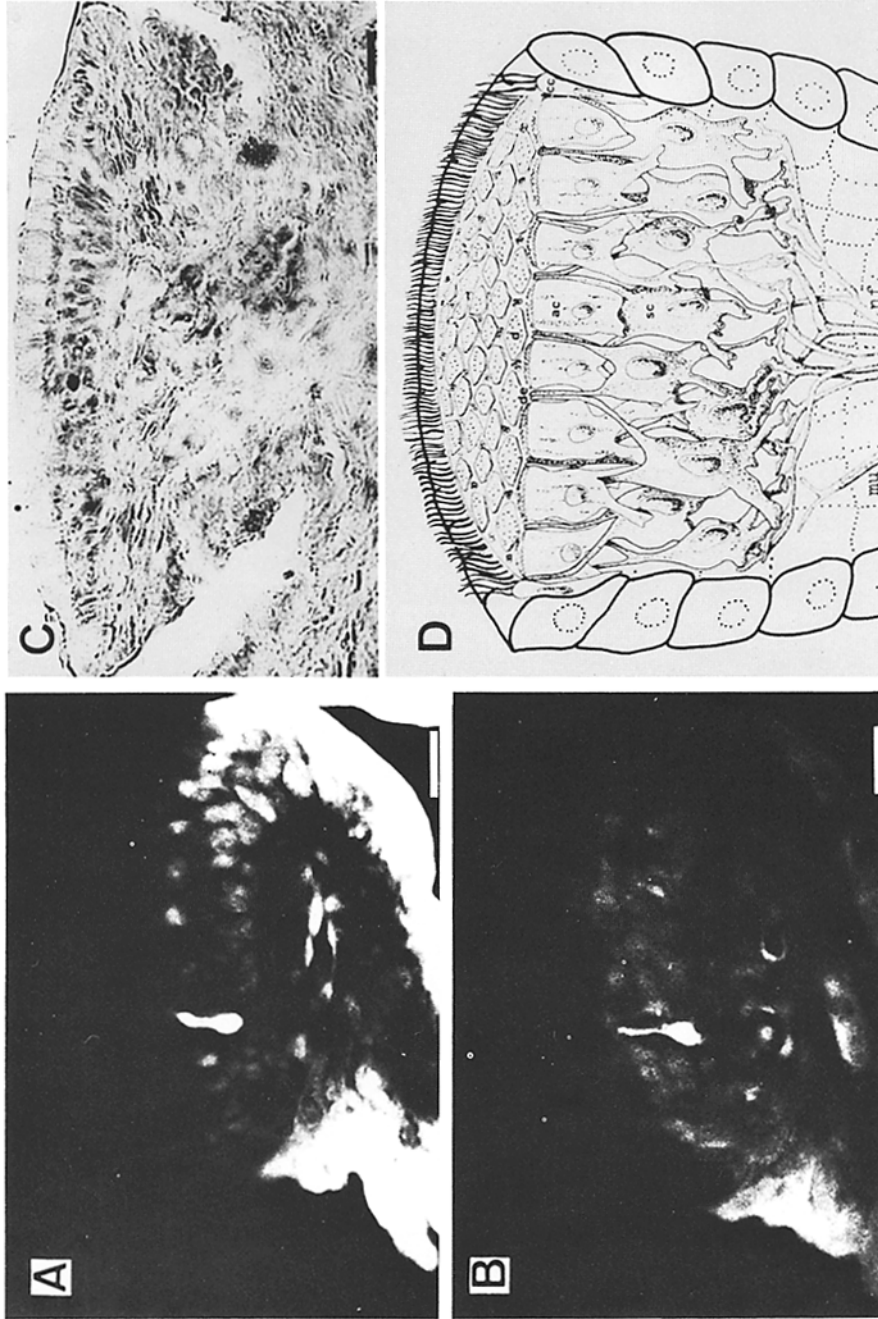
much smaller than those produced by high NaCl concentrations. Therefore, this type of taste cell was referred to as a modified type of the NS cells which was found in only two cases. On the contrary, Fig. 3 B shows that the WS cells were depolarized by water and NaCl less than approximately 0.1 M, and were hyperpolarized by NaCl greater than 0.1 M. The depolarizations and hyperpolarizations were also graded with stimulus concentrations. Usually the WS-cell depolarizations to water stimuli were much smaller than the NS-cell depolarizations to 1.0 M NaCl.

It has been demonstrated that the sensitivity of taste cells to gustatory stimuli is different from one cell to the next (Kimura and Beidler, 1961; Tateda and Beidler, 1964; Ozeki and Sato, 1972; Sato, 1969, 1972). In this study it was also confirmed that the sensitivity to water or NaCl differed among taste cells. Thus, concentration-response curves as shown in Fig. 3 were different among cells. However, the resting potentials were widely distributed, so that it is not valid to determine relative sensitivities among cells by directly comparing the amplitudes of receptor potentials obtained. Comparison of the sensitivities might be performed by normalizing the amplitude of receptor potentials. As will be described in the section that follows (see Fig. 9), receptor potential amplitudes to water and NaCl are considered to be roughly proportional to the resting potentials. Therefore, the receptor potentials obtained under various resting potentials are normalized by dividing the former potential by the latter. This normalization revealed that in both NS and WS cells the amplitudes of depolarizing responses were approximately proportional to the hyperpolarizing responses, indicating that a taste cell of high sensitivity produced large depolarization and hyperpolarization.

Histological Identification of Taste Cells

In histological sections of the frog taste disk, two types of cells, supporting cells and taste cells, are easily distinguished by different cell shape and different location of cell nuclei (Fig. 4 D). Histological investigation indicates that the supporting cells might not be in functional contact with the sensory nerve fibers, but that the taste cells establish synaptic contact with the sensory fibers (Graziadei and DeHan, 1971). Therefore, the criteria for histological identification of whether an intracellularly recorded and stained cell was a taste cell or a supporting cell were based on the morphological features.

The dyes were injected into 22 fungiform papillae after intracellular recordings of the NS-cell responses and the stained marks were observed in 11 papillae. Single taste cells alone were clearly stained in six of these papillae. In four papillae two taste cells were stained together, where one cell was stained strongly the other being stained faintly, suggesting a microelectrode penetrated the former. In the remaining papilla both a supporting cell stained



faintly and a taste cell stained strongly were found together. No supporting cell alone was stained. No substantial difference was found in the NS-cell receptor potentials recorded from these 11 papillae. Photomicrographs of two sections of the fungiform papillae in Fig. 4 A and B illustrate intracellular Procion Yellow stainings of single taste cells of NS type, which were depolarized by 0.5 M NaCl and hyperpolarized by water. It is seen that the cell body and dendrite-like process of a taste cell are clearly stained across the taste disk. When two taste disks of Fig. 4 A and B are compared with a diagram of the frog fungiform papilla of Fig. 4 D which was taken from Graziadei and DeHan's article (1971), it is clear that the stained cells are identified as taste cells.

On the other hand, after intracellular recordings of WS-cell responses, dyes were injected into eight fungiform papillae and in three papillae single stained taste cells were found. The photomicrograph of Fig. 4 C shows intracellular Niagara Sky Blue staining of a single taste cell of WS type, which was depolarized by water and hyperpolarized by 0.5 M NaCl. The taste cell body alone is stained.

Although it is not certain why two cells or more were stained, there are two possibilities: (a) There is physiological interaction between taste cells or between taste cell and supporting cell, so that Procion Yellow dye injected into a cell diffused into neighboring cells through the cell membranes. (b) The microelectrode penetrated a cell by damaging neighboring cell membranes and the current for dye injection further damaged both the cell membranes. Consequently, an injected dye into the cell diffused into the neighboring cells through the damaged membranes.

Relation between Stimulus Concentration and Resistance Change

Fig. 5 illustrates intracellular records of the membrane resistance changes accompanying the depolarizations of NS cell (B) and WS cell (C) and the hyperpolarizations of NS cell (A) and WS cell (D). In either polarity of the receptor potentials, membrane resistances changed progressively after stimulus application. These changes reached maximum at the peak and during the

FIGURE 4. (*Opposite*) Histological identification of taste cells in the frog fungiform papillae. (A) and (B) Two papillae showing a single taste cell of NS type injected with Procion Yellow after recording of NS-cell responses as in Fig. 1 A. The cell body and dendrite-like process of a taste cell are stained. (C) A papilla showing a single taste cell of WS type injected with Niagara Sky Blue after recording of WS-cell responses as in Fig. 1 B. The taste cell body alone is stained. (D) A diagram of the frog fungiform papilla cited from Graziadei and DeHan's paper (1971) by permission of the publishers S. Karger AG, Basel. (*ac*) Supporting cells which are called associate cells by Graziadei and DeHan. (*sc*) Taste cells which are called sensory cells by Graziadei and DeHan, (*d*) domes of the supporting cells, (*de*) tips of the dendrites, (*nf*) nerve fibers, (*mu*) muscle fibers, (*cc*) ciliated cells. Calibration bar, 20 μ m.

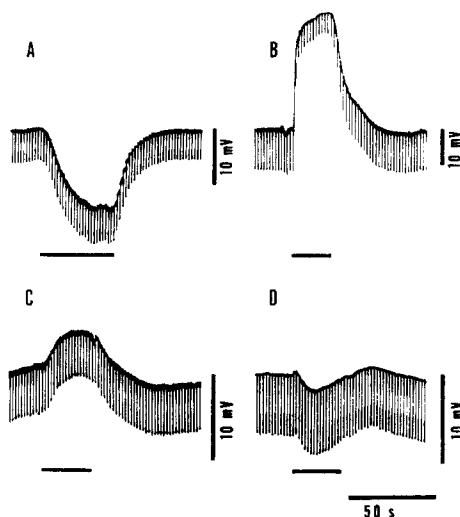


FIGURE 5. Changes in membrane resistances of taste cells during depolarizing and hyperpolarizing receptor potentials. (A) NS-cell hyperpolarizing response produced by water. (B) NS-cell depolarizing response produced by 1.0 M NaCl. (C) WS-cell depolarizing response produced by water. (D) WS-cell hyperpolarizing response produced by 1.0 M NaCl. Each record was obtained from four different cells. In each record a train of electrotonic potentials induced by constant hyperpolarizing current pulses across taste cell membrane are superimposed on resting and receptor potentials for measuring resistance changes. In A and D increment in electrotonic potential sizes after taste stimuli reflects an increase in membrane resistances, and in B and C decrement in those reflects a decrease in membrane resistances. Resting potentials in records A, B, C, and D were -12 , -20 , -23 , and -10 mV, respectively.

steady phase of receptor potentials and returned to the control level at rest after rinsing. Since the depolarizing responses of the WS cell were usually smaller than those of the NS cells, a decrease in the resistance of the WS-cell membranes was much smaller than the decrease in the NS-cell membrane resistance. For example, at the peak of depolarization in the NS cell of Fig. 5 B the resistance decreased greatly to 50% of the control measured during Ringer flow, whereas in the depolarized WS cell in Fig. 5 C the resistance decreased slightly to 92% of the control. As shown in Fig. 5 A and D, in contrast, a small increase in the resistances was observed during hyperpolarizing responses: the maximal resistance at the peaks was 109% (in the NS cell in Fig. 5 A) and 106% (in the WS cell in Fig. 5 D) of individual controls.

Fig. 6 shows the relationships between the stimulus concentrations of NaCl and the membrane resistance changes in the NS cells (A) or the WS cells (B) of three different sensitivities. Ordinates are expressed as percent of the control input resistance of each Ringer-adapted cell. As shown in Fig. 6 A, the resistance of the NS cells increased gradually with descending concentrations from 0.1 M NaCl to water, which gave rise to the hyperpolarizing re-

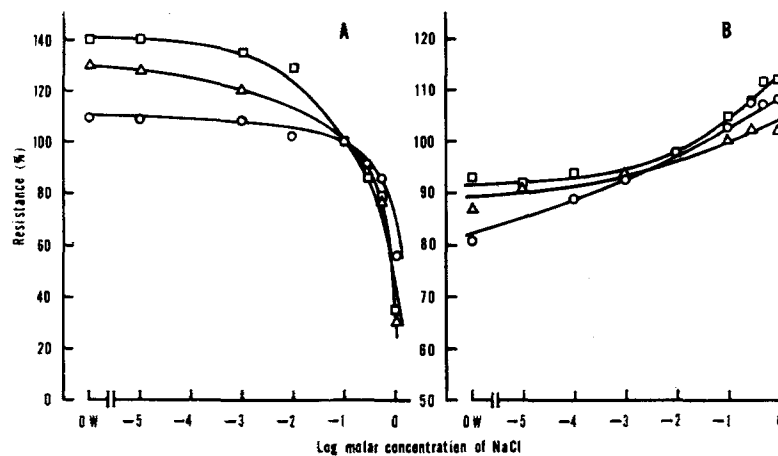


FIGURE 6. Relations between stimulus concentrations of NaCl and membrane resistance changes measured at peak responses, (A) from three different NS cells, (B) from three different WS cells. Resistances (ordinates) are expressed as percentages of control input resistance value of each cell measured during Ringer application. Resting potentials of the NS cells (A) shown by squares, triangles, and circles were around -19 , -15 , and -20 mV, respectively, and resting potentials of the WS cells (B) shown by squares, triangles, and circles were around -18 , -16 , and -21 mV, respectively.

sponses as in Fig. 3 A, and decreased largely with ascending concentrations above 0.1 M NaCl, which gave rise to graded depolarizing responses. The concentration-resistance change curves were dependent on taste cell sensitivity: the more sensitive the larger the observed resistance change. Generally, when the resistance decrease during depolarizations of a cell was large, the resistance increase during hyperpolarizations of the same cell was also large. In Fig. 6 B, the concentration-resistance relations of the WS cells of three different sensitivities are shown. It is seen that, in contrast to the NS cells, the decrease in the resistance was caused by water and NaCl concentrations of approximately less than 0.1 M, which produced depolarizing responses, whereas the increase in the resistance was caused by NaCl concentrations greater than 0.1 M, which produced hyperpolarizing responses. Table I shows means and standard deviations of resistance changes of 20 NS cells and 5 WS cells in response to water and varying NaCl concentrations.

Fig. 7 illustrates the relationships between receptor potential amplitudes produced by water and NaCl and the resistance changes in the NS cells (A) and the WS cells (B) of three different sensitivities. It is seen in Fig. 7 A that the resistance of the NS cells was greatly reduced during graded depolarizations and was slowly increased during graded hyperpolarizations. It should be noted that the receptor potential-resistance relations of the NS cells were not linear. On the other hand, these relations of the WS cells in Fig. 7 B were approximately linear in two of these cells, but not linear in

TABLE I
RESISTANCE CHANGES OF TASTE CELLS TO VARYING
CONCENTRATIONS OF NaCl

Cell	Molar concentrations of NaCl						
	Water	10 ⁻⁵	10 ⁻³	10 ⁻¹	0.3	0.5	1.0
	%	%	%	%	%	%	%
NS cells	112±14 (100-140)	110±13 (100-140)	109±12 (100-135)	100±0 (100)	90±8 (75-100)	77±17 (37-100)	54±21 (18-95)
WS cells	92±8 (81-100)	95±4 (91-100)	97±3 (93-100)	102±3 (100-108)	103±4 (100-108)	104±5 (100-112)	104±6 (100-112)

Resistances are presented as a percentage of control input resistances. Values are mean ± SD from 20 NS cells or 5 WS cells. Ranges are given in parentheses.

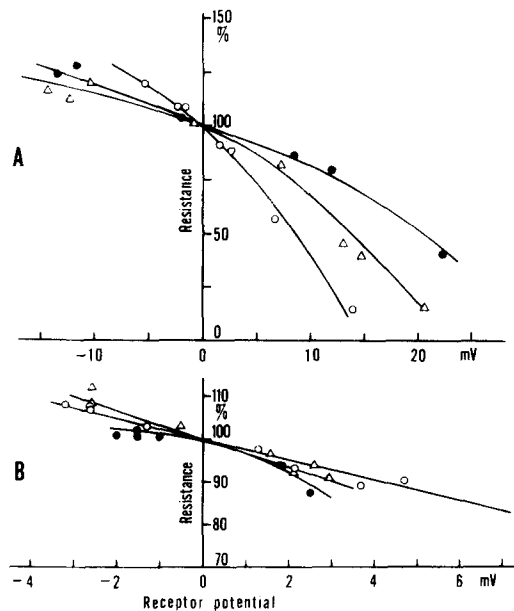


FIGURE 7. Relationships between receptor potential amplitudes and resistance changes in NS cells (A) and WS cells (B). In either A or B the relationships in three different cells (closed circles, triangles, and open circles) are plotted. In abscissa of A, positive millivolts represent depolarizing receptor potentials produced by NaCl concentrations larger than 0.1 M, and negative millivolts represent hyperpolarizing receptor potentials produced by water and NaCl concentrations less than 0.1 M. In abscissa of B, positive millivolts denote depolarizations elicited by water and NaCl solutions less than 0.1 M, and negative millivolts denote hyperpolarizations elicited by NaCl concentrations greater than 0.1 M. Resistances (ordinates) are represented as percent of control of each cell measured during Ringer flow. Resting potentials of NS cells (A) exhibited by symbols of closed circles, triangles, and open circles were around -18, -21, and -17 mV, respectively, and resting potentials of WS cells (B) exhibited by symbols of closed circles, triangles, and open circles were around -20, -18, and -21 mV, respectively.

the other cell. These linear-like relations are attributable to a narrow range of depolarizations. If the depolarizing responses of the WS cells are large enough, the relation seems not to be linear like that of the NS cells. Table II gives means and standard deviations of resistance changes of NS and WS cells under various magnitudes of receptor potentials.

Reversal Potentials

In order to measure the reversal potential or the equilibrium potential associated with the ionic mechanism of the receptor potentials, the resting membrane potentials were changed by applying inward or outward currents on the order of 10^{-10} A across the taste cell membranes. Then, a change in the amplitude and the polarity of the receptor potentials was observed during gustatory stimuli. Fig. 8 illustrates an example of the responses to 0.5 M NaCl of a NS cell under an influence of applied currents. In record A of Fig. 8, no extrinsic current was applied, the resting potential and receptor potential being at -13 and $+11$ mV, respectively. It is seen that the depolarizations to 0.5 M NaCl were gradually reduced with decreasing membrane potentials (Fig. 8 B, C) and that the polarity of the receptor potentials finally reversed when the membrane potential reached a few millivolts positive level above the zero membrane potential (Fig. 8 D, E). Fig. 9, which was plotted from the experiments as in Fig. 8, shows the relationships between the membrane potentials (abscissae) and the receptor potentials (ordinates) in two different NS cells (A, B) and two different WS cells (C, D). It is seen that the hyperpolarizing responses, which were produced by water in the NS cell (Fig. 9 A) and by 0.5 M NaCl in the WS cell (Fig. 9 D), were increased in amplitude as the membrane potentials were displaced from the resting level in the negative direction. In contrast, the hyperpolarizations were gradually decreased as the membrane potentials were displaced in the positive direction and the reversal point of the response polarity was estimated by extrapolating a straight line obtained through the experimental points. The reversal potentials so measured for the hyperpolarizing responses were about $+2$ mV for Fig. 9 A and $+8$ mV for Fig. 9 D. On the other hand, graphs B and C of Fig. 9 illustrate the membrane potential-response relations in depolarizing responses of the NS cell (B) to 0.5 M NaCl and of the WS cell (C) to water. The reversal potentials for these cells were $+7$ and 0 mV, respectively. Table III summarizes means and standard errors of the reversal potentials obtained for hyperpolarizing and depolarizing responses of both types of cells: there were all positive values of mean $+1.3$ – $+4.5$ mV.

Tateda and Beidler (1964) first demonstrated an existence of the reversal potential for rat taste cell responses to NaCl stimuli. The reversal potential values for the depolarization responses of the frog NS cells are similar to

TABLE II
RESISTANCE CHANGES ACCOMPANYING TASTE RECEPTOR POTENTIALS

Cell	Receptor potential amplitudes (mV)							
	-10.0	-5.0	-2.5	0	+2.5	+5.0	+10.0	+15.0
NS cells	119±5 (7) (115-125)	111±5 (12) (104-120)	106±3 (12) (103-114)	100±0 (12) (100)	93±4 (12) (87-98)	84±7 (12) (73-93)	62±14 (12) (41-83)	34±22 (12) (10-69)
WS cells		110±4 (3) (105-113)	103±4 (5) (100-108)	100±0 (5) (100)	91±5 (5) (88-100)	83±7 (3) (75-89)		

Resistance values are calculated from resistance-potential curves through the data points and are expressed as percent of control input resistance under Ringer adaptation. Errors are SD. Ranges are shown in lower parentheses and number of observed cells in side parentheses. The cells had resting potentials of -18 to -24 mV. Positive figures of the receptor potentials indicate depolarization and negative hyperpolarization.

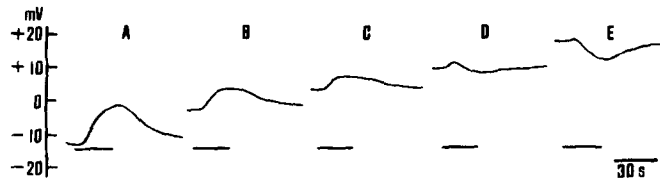


FIGURE 8. Effect of applied depolarizing currents on receptor potentials of a NS cell produced by 0.5 M NaCl. In A no extrinsic current was applied, resting potential was at -13 mV and depolarizing response was at $+11$ mV. From B to E, the resting potential was displaced gradually to positive directions by application of depolarizing current steps ranging from $+0.41$ to $+1.13$ nA. Reversal potential for depolarizing response of this NS cell was calculated to be $+7$ mV.

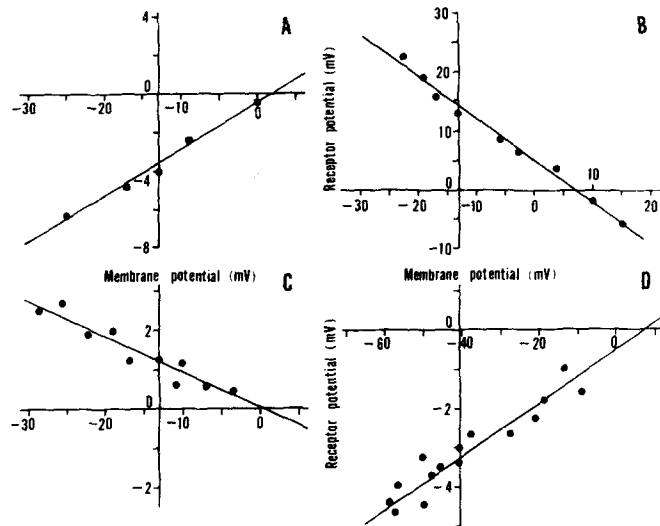


FIGURE 9. Relationships between amplitudes of receptor potentials and membrane potentials displaced by applied currents. (A) NS-cell responses to water. (B) NS-cell responses to 0.5 M NaCl, which are partly shown in Fig. 8. (C) WS-cell responses to water. (D) WS-cell responses to 0.5 M NaCl. Each graph was obtained from four different taste cells. Abscissae, membrane potential levels. Negative numerals denote negative membrane potentials. Ordinates, peak amplitudes of receptor potentials produced by water or 0.5 M NaCl. Negative numerals represent hyperpolarizing responses. Resting membrane potentials are represented by vertical axes. Resting potentials in A, B, and C were all -13 mV and resting potential in D was -41 mV. Note that reversal potentials are obtained from intersection between abscissae and straight lines through experimental points.

those found in rat gustatory cells to NaCl stimulus (Ozeki, 1970, 1971). Eyzaguirre et al. (1972) measured reversal potentials for depolarizing responses of toad taste cells to NaCl as well as other salts and obtained as high as $+40$ to $+170$ mV. These values are very much larger than our values.

TABLE III
REVERSAL POTENTIAL FOR DEPOLARIZING AND HYPERPOLARIZING
RECEPTOR POTENTIALS

Cell	Stimulus	Response	Reversal potential <i>mV</i>	No. of cells
NS cell	0.5 M NaCl	Depolarization	+4.5±0.7 (0-+7.5)	14
NS cell	Water	Hyperpolarization	+1.3±0.8 (+0.5-+2.0)	2
WS cell	Water	Depolarization	+3.4±0.8 (0-+5.0)	6
WS cell	0.5 M NaCl	Hyperpolarization	+3.5±2.4 (0-+8.0)	3

Values are means ± SE of means. Ranges are indicated in parentheses.

Effect of Na-Free Ringer on the Receptor Potentials

The evidence is accumulated that Na⁺ ions play an important role in generation of receptor potentials in some sensory cells (Diamond et al., 1958; Edwards et al., 1963; Ottoson, 1964; Brown et al., 1970). In the present experiments the effect of Na-free Ringer on the depolarizing responses of the NS cells was examined. To make Na-free Ringer 111.2 mM NaCl in normal Ringer was replaced by equimillimolar choline chloride, and NaHCO₃ and NaH₂PO₄ were not added but 0.1 mM KH₂PO₄ was employed as a buffer. In order to rapidly equilibrate the gustatory epithelium with Na-free solution, isolated tongues were used only in this series of experiments. The isolated tongues were first adapted with normal Ringer for about 30 min. Then, a microelectrode was inserted into a NS cell and the control depolarizing responses to 0.5 M NaCl was recorded (Fig. 10 Ba). When the normal Ringer adaptation was switched to the Na-free saline, the NS cell showed small depolarizations, whose amplitude was mean 2.4 ± 0.3 mV (SE, *n* = 15). The depolarizations lasted for 1.5–2.0 min and returned to the original resting level in spite of continuous flow of the Na-free Ringer. Records *b* and *c* of Fig. 10 B show intracellular depolarizing responses to 0.5 M NaCl 18 min (*b*) and 31 min (*c*) after the onset of Na-free Ringer flow. Although NaCl stimuli of 20–30-s duration were applied to the tongue surface adapted with Na-free Ringer, the NaCl stimuli did not affect the Na-free condition within the gustatory epithelium because of lack of fast diffusion of NaCl solutions through the epithelium. The aspect of response changes in two different NS cells is illustrated in the graph in Fig. 10 D. It is seen that the depolarizing responses 30 min after an onset of Na-free Ringer adaptation are reduced to around 50% of the control measured under the normal Ringer.

Usually it was difficult to hold the penetration of cells for a sufficient period of time, so that we could not observe the recovering phase of the responses

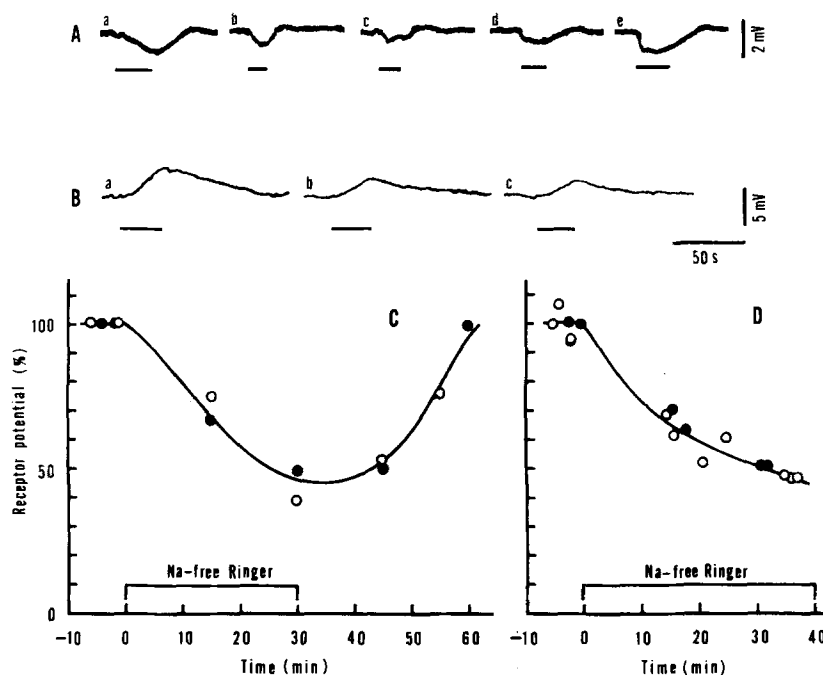


FIGURE 10. Effects of Na-free Ringer adaptation on receptor potentials. (A) Extracellularly recorded receptor potentials of a NS cell to 0.5 M NaCl. Downward deflections show negativity. (a) Control response recorded under normal Ringer adaptation. (b) Response 15 min after normal Ringer was replaced by Na-free Ringer. (c) Response 30 min after Na-free Ringer adaptation. (d) Response 15 min after return from Na-free Ringer to normal Ringer. (e) Response 30 min after the return. (B) Intracellularly recorded receptor potentials of another NS cell to 0.5 M NaCl. Upward deflections show depolarizations. (a) Control response under normal Ringer adaptation. (b) Response 18 min after Na-free Ringer adaptation. (c) Response 31 min after the adaptation. After tongues were adapted with Na-free Ringer, taste stimuli were rinsed with Na-free Ringer. Time scale shown in B is for all records of A and B. (C) Change in peak amplitudes of extracellular receptor potentials of two different NS cells (\circ and \bullet) produced by 0.5 M NaCl after Na-free Ringer adaptation and return to normal Ringer. Five points of closed circles were shown in records of A. Time 0 (abscissae) denotes onset of Na-free Ringer application and 30 min later Na-free Ringer is returned to normal Ringer. (D) Change in peak amplitudes of intracellular depolarizing receptor potentials in two NS cells (\circ and \bullet) produced by 0.5 M NaCl during Na-free Ringer adaptation. Three points of closed circles are shown in records of B. In C and D, receptor potential amplitudes (ordinates) are expressed as percent of control responses measured under normal Ringer flow. In D, resting potentials of two NS cells of open and closed circles were -8 and -10 mV, respectively.

after substituting the Na-free Ringer with the normal Ringer. Therefore, we attempted to extracellularly record the receptor potentials. In this case intracellular depolarization of the NS cell was first confirmed by applying 0.5 M NaCl and then the microelectrode inserted was withdrawn to just outside

the taste cell. Fig. 10 Aa shows the control extracellular response to 0.5 M NaCl under normal Ringer adaptation. It should be noted that the extracellularly recorded responses of NS cells were negative in polarity, which was distinguished from intracellular responses. Mean extracellular responses to 0.5 M NaCl of the NS cells under normal Ringer adaptation were -1.6 ± 0.2 mV (SE, $n = 25$) with a range of -0.4 to -4.0 mV. Records *b* and *c* of Fig. 10 A illustrate reduction of responses to 0.5 M NaCl under Na-free Ringer adaptation and the records *d* and *e* of Fig. 10 A illustrate the recovery process after a change from the Na-free Ringer to the normal. Fig. 10 C shows this time-course in two different NS cells. It is seen that the extracellular receptor potentials to 0.5 M NaCl are also reduced to about 50% of the control 30 min after Na-free Ringer flow and are mostly recovered within 30 min after return to normal Ringer. The two response curves obtained by both intracellular (Fig. 10 C) and extracellular (Fig. 10 D) recordings are very close.

Receptor Potentials under Water Adaptation

While the tongues were adapted with distilled water, NaCl concentration-response magnitude relations of the WS cells and the NS cells were examined. As expected from the effect of Na-free Ringer adaptation in Fig. 10, prolonged application of water will cause a change in composition of extracellular fluid of the tongue epithelium and will result in the change in response sizes. To eliminate this effect, concentration-response relation was measured within 10 min after normal Ringer was replaced by water. In addition, in contrast to the experiments with isolated tongues of Fig. 10, intact tongues with normal blood circulation were employed in the present series of experiments. Thus, it seems unlikely that the extracellular fluid of the gustatory epithelium is changed during water adaptation for 10 min. Fig. 11 A shows intracellular records of a NS cell under Ringer and water adaptations. This cell had the resting potential of -24 mV during continuous Ringer flow. Records *a-d* of Fig. 11 A similar to Fig. 1 A illustrate the control responses to water and to 0.1–0.5 M NaCl under Ringer adaptation. Immediately after recordings of the control responses, the Ringer flow was switched to water flow at the point arrowed in Fig. 11 Ae. As a result, the resting potential of -24 mV shifted gradually to the hyperpolarizing direction and finally became at -32 mV (the second row of Fig. 11 A). With this new membrane potential no hyperpolarizations were evoked by water and NaCl stimuli lower than 0.001 M. Under the control Ringer adaptation, this NS cell did not respond to 0.1 M NaCl (Fig. 11 Ab), whereas, under water adaptation, the cell responded to the same stimulus by producing detectable depolarization (Fig. 11 Ag). This denotes lowering of the NaCl threshold for depolarization. The amplitudes of depolarizing responses under water adaptation were always

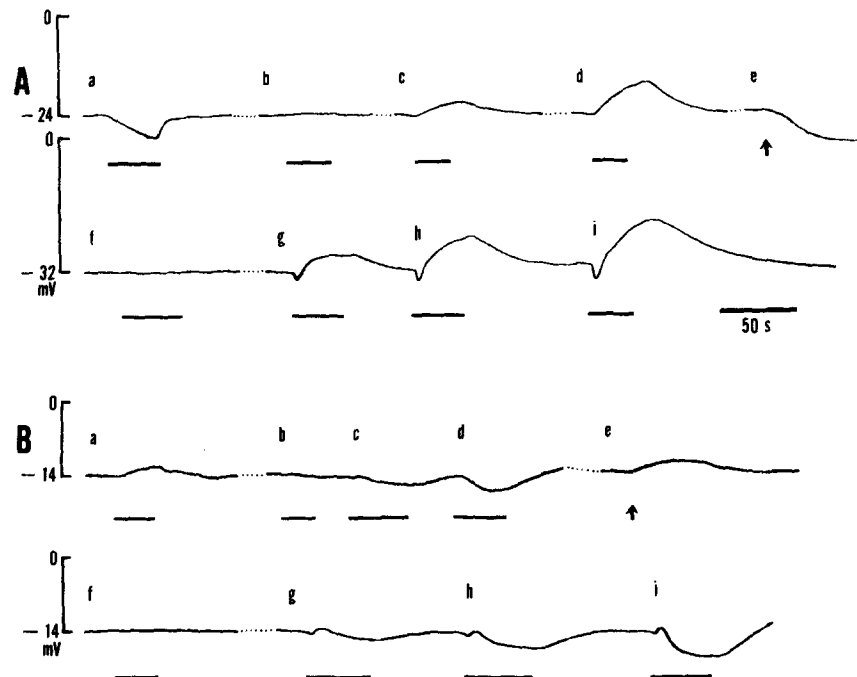


FIGURE 11. Effects of change in adapting solutions on receptor potentials. (A) From a NS cell. (*a-d*) Control responses under Ringer adaptation. (*e*) Change from Ringer adaptation to water at the point marked by an arrow. (*f-i*) Responses under water adaptation. Stimulus solutions: (*a* and *f*) Distilled water. (*b* and *g*) 0.1 M NaCl. (*c* and *h*) 0.3 M NaCl. (*d* and *i*) 0.5 M NaCl. On the left, zero and resting membrane potentials are shown. (B) From a WS cell. (*a-d*) Responses under normal Ringer adaptation. (*e*) Change from normal Ringer to water flow at the point marked by an arrow. (*f-i*) Responses during water adaptation. Stimuli: (*a* and *f*) Water. (*b* and *g*) 0.3 M NaCl. (*c* and *h*) 0.5 M NaCl. (*d* and *i*) 1.0 M NaCl. Time scale shown in record *i* of A is common for all records of A and B.

larger than those under Ringer adaptation over all ranges of concentrations above 0.1 M (Fig. 11 *Ag-i*). However, it should be noted that in the water-adapted NS cells small hyperpolarizations usually followed by large depolarizations appeared immediately after applications of NaCl larger than 0.01 M (Fig. 11 *Ag-i*). With increasing concentrations, the amplitudes of the initial hyperpolarizing responses were increased. Fig. 12 A, which was obtained from the experiment of Fig. 11 A shows the dose-response curves of the NS cell under Ringer adaptation (open circles) and under water adaptation (filled circles).

Fig. 11 B shows intracellular responses of a Ringer- or water-adapted WS cell to water and varying NaCl concentrations. The control responses are shown in the records *a-d* of Fig. 11 B, which are similar to Fig. 1 B. When the Ringer flow was replaced with water at an arrow in Fig. 11 *Be*, this WS cell

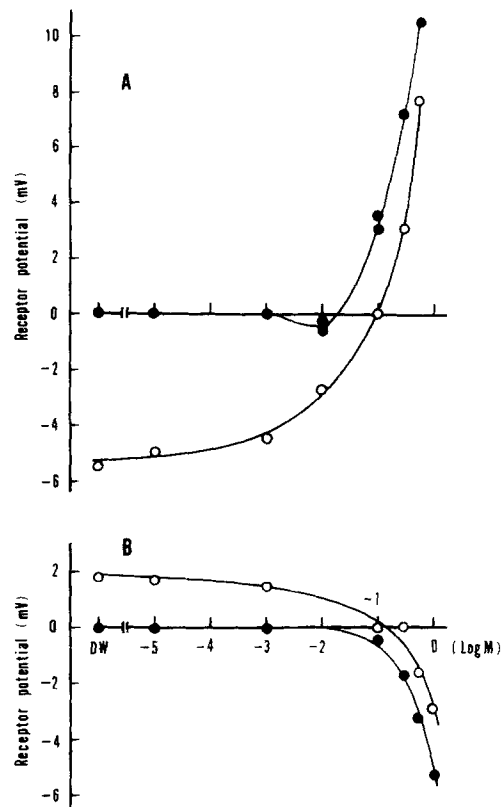


FIGURE 12. Relationships between stimulus concentrations of NaCl and peak receptor potential amplitudes under Ringer and water adaptations. (A) From responses of the NS cell partly shown in Fig. 11 A. (B) From responses of the WS cell partly shown in Fig. 11 B. In A and B open circles represent responses obtained under normal Ringer adaptation and closed circle responses under water adaptation. Abscissae, logarithm of molar concentrations of NaCl and distilled water (DW). Ordinates, peak amplitudes of receptor potentials. Negative numerals represent hyperpolarizing responses. Small hyperpolarizing responses as shown in records *g-i* of Fig. 11 A are not plotted in closed circles of graph A excepting responses to 0.01 M NaCl. Similarly, small depolarizing responses as shown in records *g-i* of Fig. 11 B are not plotted in closed circles of graph B.

was depolarized. This depolarization declined to the original resting level of -14 mV during continuous water adaptation. In spite of the same resting potential, the responses to water and NaCl changed greatly under water adaptation: no depolarizing responses were generated by water and NaCl stimuli lower than 0.001 M (Fig. 11 Bf) and graded hyperpolarizing responses larger than control responses were generated with increasing concentrations of NaCl (Fig. 11 Bg-i). The dose-response curve of this WS cell is illustrated in Fig. 12 B. Open circles denote the responses under Ringer adaptation and filled circles the responses under water adaptation. As shown

in records *g-i* of Fig. 11 B, a small depolarization was superimposed on the hyperpolarizing responses immediately after onset of stimulus applications.

DISCUSSION

Relation Between Time-Course of Taste Receptor Potentials and that of Gustatory Nerve Impulses

In the vertebrate gustatory system, no simultaneous recordings from the taste cells and fibers have been demonstrated to date. Thus, the comparison of time-courses of these responses must be done by independently recorded data. The frog taste receptor potentials obtained in the present experiments show a slow time-course having no initial transient phase: The peak time after stimulation is 15–40 s and the fall time after rinse 25–70 s. This slow temporal course derives from a stimulus application method which provides similar slow time-course of concentration change of a stimulant on the taste receptor membrane. Previous investigations performed with different stimulus presentation techniques indicate that the peak times of taste receptor potentials are 7–8 s (Sato, 1972) and 1–3 s (Akaike et al., 1973) in frogs, and 5–15 s (Kimura and Beidler, 1961) and 5–10 s (Ozeki and Sato, 1972) in rats. Nevertheless, these peak times are much longer than the peak time of 20–30 ms in the insect chemoreceptor potentials elicited by taste stimuli with a rapidly rising rate (Morita and Yamashita, 1966). In photoreceptors (Washizu, 1964) and mechanoreceptors (Nishi and Sato, 1968; Ottoson and Shepherd, 1971), it is proved that the rate of rise and the peak time of receptor potentials depend upon the rate of rise of a stimulus.

Thus, it is assumed that the time-course of taste receptor potentials is presumably also a function of the rate of rise of a stimulus. The rapid peak time similar to that of the insect chemoreceptor potentials may be obtained from vertebrate taste cells, if a square waveform of chemical stimuli is presented to the taste receptor membrane proper without disturbing intracellular recording.

On the other hand, gustatory nerve impulse discharges consist of the initial phasic component and the following tonic. Investigations of whole nerve or single fiber activities from the rat chorda tympani demonstrate that the time to the initial phasic response peak is on the order of 100–500 ms (Sakamoto, 1967; Halpern and Marowitz, 1973; Ogawa et al., 1973). Similar values are found in the frog gustatory nerve discharge (unpublished observation of T. Sato). These peak times of the phasic nerve discharges are much shorter than those of the taste receptor potentials obtained. This discrepancy seems to ascribe to different rates of taste stimulus flows which are a parameter of the rate of rise of a stimulus on the receptor membrane. The rapid flow rate of 100–5,000 $\mu\text{l/s}$ is usually employed for recording the gustatory nerve re-

sponses, while, in order to prevent the removal of an inserted microelectrode, the slow rate of 1–20 $\mu\text{l/s}$ is used for recording taste receptor potentials. The amplitude and peak time of the dynamic phase in the gustatory nerve response are a function of the rate of rise of a taste stimulus, so that a very slowly rising stimulus eliminates the initial transient phase (unpublished observation of T. Sato). The same properties are found in the neural response from other receptors (Shepherd and Ottoson, 1965; Lindblom, 1965). Therefore, it is concluded that initial transient firing rate of the taste nerve impulses is probably a function of the rate of rise of the depolarizing taste receptor potentials or subsequent excitatory postsynaptic potentials evoked in the gustatory nerve endings, and that tonic response is probably a function of the steady phase amplitude of the receptor potentials or the postsynaptic potentials. Presumably, the hyperpolarizing receptor potentials reduce the release of transmitter substance from the taste receptor terminals, resulting in decrement of gustatory nerve impulses.

Recently Faull and Halpern (1972) point out that taste receptor potentials recorded so far cannot be actual receptor potentials because the time-course of slow potentials of taste cells is much slower than that of gustatory impulse discharges. However, this hasty conclusion seems to be a misunderstanding, because the difference of the time-courses in the taste nerve and cell probably derives from different experimental methods. From this point of view, it is safe to say that the obtained depolarizing receptor potentials, even if slow, might be associated with the gustatory nerve impulses.

Mechanism of Receptor Potential Generation

In the present study the depolarizing receptor potentials of both the NS and WS cells indicate (a) accompanying decrease in taste cell membrane resistances and (b) reversal potentials of a few millivolts positivity above zero membrane potential. It is accepted that the stimulus solutions applied on the rat or frog tongue surface do not easily penetrate the gustatory epithelium (Beidler, 1967; Mistretta, 1971; Ozeki and Noma, 1972). Thus, these evidences lead us to conclude that the depolarizing receptor potentials of both cell types are associated with an increase in ionic permeability of the taste cell membranes apart from their exposed receptor membranes. It is not yet clear what ion species are involved in permeability changes. However, the present experiments reveal that the amplitudes of NS-cell depolarizations are Na^+ dependent. This strongly indicates that Na^+ ions, whose equilibrium potential is well known to be more positive than zero membrane potential, play an important role in generation of depolarizing responses of the NS cells. With the WS cells it is not determined whether their depolarizing responses produced by water are Na^+ dependent. However, the reversal potentials for depolarizing responses of both NS and WS cells are almost the

same values of mean +3.5 to +4.5 mV. Thus, it is supposed that Na⁺ ions are also related with the production of WS-cell depolarizations. It is probable that other ions such as K⁺ and Cl⁻ are concerned with the depolarizing responses. However, equilibrium potentials for K⁺ and Cl⁻ are well known to be near or more negative than resting potentials in many kinds of cells. Therefore, it is considered that Na⁺ influxes from extracellular spaces to taste cell membranes could be dominantly associated with depolarizing receptor potentials in both cell types. It should be noted that Na⁺ influxes do not originate from the NaCl stimulus applied to the tongue surface. Our conclusion concerning the ionic mechanism of the depolarizing responses to water and NaCl stimuli is consistent with that proposed by Ozeki (1970, 1971), who studied rat gustatory cells.

The present experiments indicate that under Ringer adaptation of the tongue, hyperpolarizing receptor potentials are elicited in the NS cells by water and NaCl below 0.1 M and in the WS cells by NaCl above 0.1 M. These hyperpolarizations are accompanied by a slight increase in membrane resistance and indicate the reversal potential of a few millivolts positivity above the zero membrane potential.

On the other hand, it is well known that hyperpolarizing responses of inhibitory postsynaptic potentials (IPSP's) are accompanied by a decrease in the membrane resistance, and usually show more negative reversal potential than the resting potential (Eccles, 1964). The IPSP's are postulated to result from an increased permeability to either K⁺ or Cl⁻ ions or to both ions, whose equilibrium potentials are usually near or more negative than the resting potential (Eccles, 1964). These evidences suggest the mechanism generating the hyperpolarization responses of taste cells is quite different from the mechanism of the IPSP's.

It is assumed that, during continuous Ringer adaptation, the surface receptor membranes of the NS and WS cells are constantly stimulated by some substances, probably 111.2 mM NaCl of the Ringer solution, and that the cell membranes below the receptor membranes are slightly depolarized by an increase in the membrane permeability to probably Na⁺ ions involved in the gustatory epithelium. Therefore, a mechanism producing the hyperpolarization of the NS and WS cells is probably due to an elimination of the Ringer-induced depolarizations by application of water or concentrated NaCl to the tongue. In other words, the hyperpolarizing taste responses could be attributable to a decrease in Na⁺ permeability activated slightly by the adapting Ringer. This explanation seems to support the view that the hyperpolarizing responses of the NS or WS cells are a type of disfacilitatory hyperpolarizations which are already found in vertebrate photoreceptor cells (Toyoda et al., 1969; Murakami et al., 1972) and some neurons in the central nervous system (Llinas, 1964; Toyama et al., 1968).

Mechanism of Receptor Cell Stimulation

Concerning salt receptor stimulation, Beidler (1954, 1967) has proposed (*a*) that the binding of a stimulus cation to the anionic site of taste receptor membranes presents an excitatory effect on the generation of gustatory responses and the binding of an anion to the cationic site an inhibitory effect, and (*b*) that the net magnitude of gustatory responses depends on the relative number of cationic to anionic membrane sites available to taste stimuli, and on the relative effectiveness of an excitatory cation to an inhibitory anion. Going along with these proposals we are able to explain the present results of frog taste cell responses. Since NS and WS cells generate the receptor potentials of opposite polarity in response to either water or high NaCl concentrations, and the depolarizing and hyperpolarizing responses of both cell types are probably explained by Na⁺ permeability change in the cell membranes, it is postulated that the surface receptor membranes of two taste cell types possess slightly different physicochemical properties. We hypothesize that the NS cells have more numerous anionic sites than cationic on the receptor membranes and, in contrast, WS cells have more numerous cationic sites than anionic.

Although the resting potentials of taste cells are probably maintained mainly by the difference in potassium concentrations between the inside and outside of the cell membranes, the amplitude of resting potentials may be affected by stimulating effectiveness of the adapting solutions on the receptor membranes. When the frog tongue is adapted to Ringer saline, it is presumed that the binding of the Ringer cations to the anionic receptor sites may produce a depolarizing effect on the taste cell and the binding of the Ringer anions to the cationic sites a hyperpolarizing effect. When 100 mM NaCl stimuli similar to 111.2 mM NaCl of the adapting Ringer solution are given to the NS and WS cells, the resting potentials seldom change (Fig. 3). This suggests that under a Ringer-adapted state the resting potentials of the NS and WS cells may be mainly modified by a balance of the depolarizing action of Ringer Na⁺ and the hyperpolarizing action of Ringer Cl⁻. The other cations of K⁺ and Ca⁺⁺ and the anions of HCO₃⁻ and H₂PO₄⁻ are considered to be of lesser importance in the Ringer.

With NS cells the resting potential under Ringer adaptation is smaller than that under water adaptation (Fig. 11 A). This suggests that the resting potential of Ringer-adapted NS cells is decreased by the Na⁺ binding to their anionic receptor membrane sites, resulting in maintenance of a depolarization. The Cl⁻ binding to the cationic sites of the NS cells may play a lesser role in the resting potential. When stimulating the NS cells by concentrated NaCl, the surface receptor membranes, which are composed of more numerous anionic sites than the cationic, may receive numerous bindings of

stimulus Na^+ and few bindings of the Cl^- . Thus, the NS cells show depolarizing receptor potentials. On the other hand, stimulation of the NS cells with water and NaCl below 0.1 M would reduce the number of the Ringer Na^+ -anionic site complexes, resulting in the production of hyperpolarizing receptor potentials. The present experiment (Figs. 11 A and 12 A) indicates that under water adaptation of the NS cells no hyperpolarizing responses are generated by stimulation with water to 0.001 M NaCl, but small graded hyperpolarizations followed by prominent depolarizing responses are generated by NaCl stimuli above 0.01 M. Since the receptor membranes of water-adapted NS cells are not bound by any cations and anions, it is assumed that the initial small hyperpolarizations may be produced by the initial effective binding of the stimulus Cl^- to the cationic membrane sites of the NS cells, because an ionic mobility of Cl^- is faster than that of Na^+ .

With respect to the WS cells, which are hypothesized to possess more numerous cationic sites than anionic, it is likely that during the Ringer adaptation the cationic sites of the WS cells are dominantly bound with Ringer Cl^- . By the proposed inhibitory action of the Cl^- ions the membrane potential of the WS cells may be slightly hyperpolarized rather than under water adaptation, but may be more depolarized than under concentrated NaCl adaptation. When water and NaCl stimuli below 0.1 M are applied to the Ringer-adapted WS cells, Ringer Cl^- -cationic site complexes will be reduced in number, resulting in depolarizing responses. In contrast, when NaCl stimuli above 0.1 M are applied, the stimulus Cl^- ions would show a powerful inhibitory effect by filling numerous cationic sites and the WS cells are thereby strongly hyperpolarized. The present experiments (Figs. 11 B and 12 B) indicate that the application of NaCl stimuli above 0.01 M to the water-adapted WS cell produces hyperpolarizing responses, which are immediately followed by small depolarizing responses. Since a mobility of Cl^- ions is faster than that of Na^+ ions, initial prolonged hyperpolarizations would be produced by initial inhibitory effect of stimulus Cl^- ions on the cationic sites and subsequent small depolarizations would be produced by later excitatory effect of stimulus Na^+ ions on the poor anionic sites. In conclusion, the mechanism underlying different response production in two types of taste cells, NS and WS cells, might be due to different ratios of cationic to anionic membrane sites.

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