Intracellular Characteristics and Responses of Taste Bud and Lingual Cells of the Mudpuppy

CHARLES H. K. WEST and RUDY A. BERNARD

From the Department of Physiology, Michigan State University, East Lansing, Michigan 48824. Dr. West's present address is Department of Neurophysiology, University of Wisconsin Medical School, Madison, Wisconsin 53706.

ABSTRACT Intracellular recordings of membrane potentials of mudpuppy lingual cells were made with micropipette electrodes. Three types of cells were distinguished by their responses to chemical stimulation. Surface epithelial (SE) cells outside of taste buds responded with large membrane potential and resistance changes to a variety of stimuli representing the four taste qualities. Salts and acids evoked particularly large potential changes, and MgCl₂, acids, and quinine greatly increased the membrane resistance. One type of taste bud cell (TB-1) was characterized by large depolarizations to K salts, and the other type of taste bud cell (TB-2) characteristically hyperpolarized to MgCl₂, acid, and sugar solutions. Membrane resistance changes accompanying TB-1 and TB-2 cell responses were relatively small compared to those of SE cells. Electrotonic coupling was observed between pairs of SE and TB-2 cells but not for pairs of TB-1 cells nor cells of different types. After recording cell responses, dye-marking allowed verification in situ and histologically. From the identification of cells in section, it is hypothesized the TB-1 and TB-2 cells correspond to light and dark cells, respectively. Responses of TB-1 cells imply a taste receptive function; whereas TB-2-cell responses suggest secretory, supportive, and (or) receptive functions. Factors affecting cellular characteristics, non-taste bud cell responsiveness, response mechanisms, and function of electrotonic coupling are discussed in relation to taste reception.

INTRODUCTION

Gustatory reception generally is believed to occur within the taste buds because of their location and distinct morphology as differentiated epithelial cells contacting both lingual surface and nerve fibers (see review by Murray, 1971). Responses to chemical stimuli recorded intracellularly from taste bud cells of rats and hamsters (Kimura and Beidler, 1961; Tateda and Beidler, 1964; Ozeki, 1970, 1971; Ozeki and Sato, 1972) and taste disc cells of frogs (Sato, 1969, 1972, 1976; Sato and Beidler, 1973, 1975; Akaike et al., 1973, 1976; Akaike and Sato, 1975, 1976a, b) were assumed to be gustatory receptor potentials transduced by these cells. Their magnitude and time-course depended upon stimulus quality, concentration, rate of onset, and other factors; the change in membrane conductance that often accompanied these "receptor potentials" reflected the
various generating mechanisms of different stimulus qualities. When Eyzaguirre et al. (1972) recorded from cells of isolated toad lingual mucosa, all surface cells penetrated, regardless of location, responded to taste stimuli. These results raised questions about the degree of specialization of taste organ cells and suggested that other nonkeratinized lingual cells might contribute to gustatory input, at least in some species.

Recent studies of taste bud morphology have revealed features possibly of functional significance requiring physiological investigation. Classically, mature taste bud cells are categorized into light or dark cells by their structure. After studying rabbit taste bud ultrastructure, Murray (1971) suggested that the light cells are the receptor elements and that the dark cells have secretory, supportive, and (or) sensory function (contrary to most previous conclusions), and Farbman and Yonkers (1971) agreed with this functional distinction for taste bud cells in the mudpuppy. However, no physiological evidence for association of structure and function has been reported. Also, tight (gap) junctions observed between taste organ cells in several species including the mudpuppy (Farbman and Yonkers, 1971; Stensaas, 1971) may be sites for electrotonic coupling which could affect taste reception.

In this study, electrical characteristics and responses to chemical stimulation of taste bud and non-taste surface cells of the mudpuppy lingual epithelium were compared by using intracellular recording techniques. These cells also were tested for electrotonic coupling, and evidence is given from dye-marking of recorded cells for a correlation of structure and response of the two taste bud cell types.

MATERIALS AND METHODS

Preparation
Experiments were performed on adult mudpuppies (Necturus maculosus) of 15–30 cm in length that were maintained at low temperature in filtered, aerated water containing about 25 mg/liter tetracycline. Animals were anesthetized until movement of the gills ceased, either by submersion in a 5% solution of urethane or by a 1.5-2.5-ml i.p. injection of 20% urethane, with the final level carefully reached by dripping urethane solution over the gills. This later method proved more successful in maintaining proper lingual circulation. The animal was kept moist and adapted to room temperature (23–25°C) for > 1 hr before recording. During the experiment, the animal was secured, and its mouth was held open to allow access to the nondistendible tongue.

Gustatory Stimulation
Tongues were adapted to a constantly flowing solution of 0.1 M NaCl in most experiments. This saline was preferred as a rinse over pure water because it allowed the resting potentials of surface cells to be measured with greater recording stability. The normal NaCl concentration for the mudpuppy tongue (i.e., its mucous covering) is unknown, but 0.1 M NaCl compares well with the 0.111 M NaCl in amphibian Ringer solution used by some workers but with the advantages of no cell shrinkage and simplicity (for relating to stimuli and minimizing effects of adaptation).

The salt solutions used as stimuli included the following range of concentrations, in half-log steps: 0.003–1.0 M NaCl; 0.003–0.3 M KCl; 0.03–0.3 M Na2SO4; 0.01–0.5 M K2SO4; 0.03–0.3 M NH4 Cl; 0.01 and 0.1 M KI; 0.03–0.1 M MgCl2. All salts, including the
adapting NaCl, were dissolved in deionized water with resistivity > 2 MΩ/cm. Some preliminary tests with 0.1 M CaCl₂ caused enormous and long-lasting increases in system resistance that appeared to alter subsequent responses to other stimuli; therefore, tests with this salt were not continued. Other stimuli were quinine (0.003 and 0.03 M QHCl and 0.003 M QSO₄); 0.0003, 0.001, and 0.003 M H₂SO₄ and HCl; and 0.1 and 0.3 M sucrose and fructose. All of these non-salt stimuli were made in 0.1 M NaCl adapting fluid. The pH for all solutions generally ranged from 6 to 7 except for the acid solutions, which were between pH 4 and 5. Amino acids were tested in nerve recording experiments in our lab producing only poor, transient responses and, therefore, were not used for these experiments.

To avoid any mechanical disturbance that might dislodge an electrode, a gravity-fed flow-system was designed allowing a constant flow of test or adapting solution at a rate of about 4.6 ml/min through a small chamber over the tongue (Fig. 1). The switching valve in this system permitted rapid exchange between adapting and stimulating solutions without mechanical artifacts. Switching involved progressive but rapid reversal of the connections between the two inputs (stimulating and adapting solutions) and outputs (to tongue and to waste container) of the valve with minimal mixing of the two solutions. The arrival of a new solution at the recording site could be estimated to within 1 s.

Recording Apparatus
Glass pipette microelectrodes with outer tip diameters < 0.5μm (confirmed with SEM) were pulled from capillary tubing containing a fine glass fiber fused to the inner wall.
(Frederick Haer & Co., Ann Arbor, Mich.) to facilitate filling the electrode to the tip. Electrolyte solutions were injected through a Swinney filter apparatus for ultrafiltration connected to a fine needle which was inserted into the stem end of the electrode. Most of the electrodes were filled with a solution of 10% Procion navy blue dye H3R with 3% sodium bicarbonate which produced resistance ranging from 60 to 100 MΩ. Electrodes filled with 2.7 M KCl used in a few experiments had resistances roughly half that with the dye solution but yielded essentially the same experimental results. Electrodes with initial resistances greater than 100 MΩ or tip potentials > -30mV were not used. The indifferent electrode was a short piece of tubing containing Ringer agar that contacted the moist skin on the animal's head.

Both active and indifferent electrodes were connected to holders (W-P Instruments, Inc., New Haven, Conn.) containing a Ag-AgCl half-cell and filled with 2.7 M KCl. Signals were led into two high-impedance, capacity-compensated DC preamps (W-P Instruments, Inc., models M4-A and 750) and then were displayed on a dual-beam storage oscilloscope (Tektronix, Inc., Beaverton, Ore., model 5103 N) and recorded on a polygraph (Gilson Medical Electronics, Inc., Middleton, Wis., model ICT-5H with model IC-MP amplifiers). To continuously monitor the resistance during recording, hyperpolarizing constant current pulses of 0.1 nA were injected through the electrode at a frequency of 1 Hz by means of a bridge circuit in the model M4-A. Each mV of potential deflection corresponded to a 10 MΩ resistance of the electrode plus the cell membrane, and subtracting the known electrode resistance from this left cell membrane resistance. In a few experiments, electrode resistance was balanced by the bridge circuit so that only the membrane resistance was monitored.

**Impalement of Cells**

Once a taste bud was located within one of the small eminences on the mudpuppy tongue, the electrode gradually was lowered into the tissue. Cell penetration was signaled by an abrupt negative drop in the potential, accompanied with increased resistance. For recordings from deeper cells, several potential deflections were noted as the tip of the electrode passed through the more superficial cells, and the total deviation from zero potential was considered the resting potential of the deeper cell. For taste bud cells, similar values were obtained by penetration directly at the surface or obliquely through surrounding tissue. Only cells that retained at least 80% of this potential for more than a minute after penetration were subsequently used for chemical testing.

Recordings with high resistance electrodes are susceptible to physicochemical artifacts generated at the electrode tip when outside a cell, and changing the solution bathing the cells and the electrode complicates this problem. To control for the possible effects of similar artifacts during intracellular recordings the following observations were made: (a) Artifact potential changes (APC) and associated resistance changes evoked by switching from 0.1 M NaCl to test solutions were compared to cellular responses, revealing several significant differences. Several of the larger differences are listed for surface epithelial (SE) cells because many of their responses resembled APC. For example, Na2SO4 and K2SO4 both gave hyperpolarizing APC even at 0.3 M; whereas SE cells gave depolarizations often to 0.1 M, and always to 0.3 M solutions. The abrupt hyperpolarizing APC to QHCl differed from the slow depolarization or no potential change frequently recorded from cells. Sucrose solutions caused no APC, but they evoked receptor potentials in many SE cells. The large increase in membrane resistance seen in SE cells to 0.1 M MgCl2 was in direct contrast to the decrease of system resistance associated with the APC. SE cells responded to H2SO4 solutions with large potential changes that were slow to decay and with large increases in membrane resistance, but the APC to acid solutions were small (< 10 mV) and quick to decay, with slight decrease in
system resistance at the higher concentration. Differences between APC and taste bud (TB) type TB-1 and TB-2 cell responses were so obvious that no additional proof of their validity is needed. (b) Responses were reduced or absent in deep epithelial cells or decaying preparations. (c) Dye injected in cells after recording was localized within the cells when seen in section.

Dye-Marking and Histology

Procion navy blue dye was iontophoretically injected into a cell after recording, usually by a large pulse of constant hyperpolarizing current (in microampere range) applied to the electrode for 1-5 min. When the dye in the electrode occluded the tip, the clog could be broken in some cases by briefly reversing the direction or increasing the magnitude of the current.

After dye injection, the tongue was fixed with either a 6% glutaraldehyde or an acid formalin solution. Dye often would diffuse from the cell during glutaraldehyde fixation, but this problem was eliminated by using an acidic (pH about 5) formalin fixative consisting of the following: stock formaldehyde (40%) solution, 10%; ethanol, 45%; glacial acetic acid 5%; and deionized water, 40%. The tissue containing the dyed cell was fixed for 1-2 h and then stored in cacodylate buffer solution until it was histologically processed. After being dehydrated with acetone and embedded in epon, 5-7-μm thick sections were cut.

RESULTS

Electrical Properties of Lingual Cells

The responses obtained by intracellular recording from taste bud and general epithelial cells of the mudpuppy tongue were sufficiently different to distinguish at least three types of cells. The cell categories are surface epithelial (SE) cells, which are non-taste bud elements, taste bud cells (TB-1), outstandingly sensitive to K salts, and other taste bud cells (TB-2), giving many responses of opposite polarity from those of the other two cell types.

The resting potential, as measured shortly after penetration, for SE cells adapted to 0.1 M NaCl was $-20.1 \pm 1.1$ mV (mean ± SE, $n = 67$) with an input resistance of $26.5 \pm 2.7$ MΩ ($n = 39$). Under the same conditions, resting potential values for TB-1 and TB-2 cells were $-39.4 \pm 1.6$ mV ($n = 51$) and $-36.2 \pm 2.0$ mV ($n = 21$), respectively. Input resistance was $28.6 \pm 2.7$ MΩ for 28 TB-1 cells and $23.6 \pm 2.9$ MΩ for 14 TB-2 cells. The differences between resting potentials of SE cells and both types of taste bud cells were statistically significant ($P > 0.001, t$ test). However, no significant difference was found between the resting potentials of the TB-1 and TB-2 cells nor between the input resistances of all three types of cells ($P > 0.1$).

By assuming that the general epithelial (including SE) cells are nearly spherical in shape with an average cell diameter of 10 μm (personal observations), the total membrane area of these cells was approximated at $3.14 \times 10^{-4}$ cm². This value times the mean input resistance of these cells gives an areal specific membrane resistance of $\sim 8.3$ kΩ cm². Inasmuch as taste bud cells have significantly larger surface dimensions but similar input resistances, their areal specific membrane resistances would be larger than that of general epithelial cells. The relationship between applied current and resultant membrane potential change was nearly linear for all three types of cells for the range of currents.
tested, except for some decrease in slope for the TB-2 cells at hyperpolarizations of > 20 mV (Fig. 2).

Responses of Lingual Cells to Salts and Water

Applying various salt solutions to the tongue caused membrane potential changes of every SE cell examined. These potential changes resembled responses to chemical stimulation reported for taste cells, although these cells are not generally considered receptors.

Typically, for the monovalent cationic salts tested, the SE cells would depolarize to solutions more concentrated than the adapting 0.1 M NaCl and hyperpolarize to more dilute solutions and water (Fig. 3). These responses were quite regular, beginning with a rapid potential change during the first several seconds of stimulation and then plateauing to a rather constant and maintained level. Their amplitude was proportional to the difference in concentrations of adapting and test solutions. Depolarizations, whether rising or falling phase of the response, tended to be more rapid than the hyperpolarizations of that response. The large hyperpolarizations (often 50 mV or more) evoked by 0.008 M NaCl, KCl, or water were not well maintained, gradually decreasing in an irregular manner. All three cell types gave an additional brief (1-10 s) hyperpolarization of up to 25 mV at the offset of a water stimulus, followed by a quick return to the resting potential (Fig. 4).

Responses of SE cells to NaCl and KCl were very similar (Fig. 3 A), but responses evoked by NH₄Cl, Na₂SO₄, and K₂SO₄ were more variable (Fig. 5). For example, solutions of 0.1 M Na₂SO₄ and K₂SO₄ would cause a small (7 mV or less) or no potential change of either polarity. Higher concentrations of NH₄Cl
were particularly effective in depolarizing SE cells, with 0.1 M frequently causing a small depolarization and 0.3 M depolarizing these cells more than the same concentrations of other salts. Occasionally, 0.03 M NH₄Cl would evoke a small, brief depolarization followed by a repolarization to near or slightly below base line. The only divalent cationic salt extensive tested, MgCl₂, depolarized SE cells always at 0.1 M and sometimes at 0.03 M. The effectiveness series for depolarization of SE cells by 0.1 or 0.3 M salts was MgCl₂ > NH₄Cl > NaCl = KCl > Na₂SO₄ = K₂SO₄.

![Graph showing responses recorded from SE cells to solutions of various salts](image)

**Figure 3.** Responses recorded from SE cells to solutions of various (A) 0.3M or (B) 0.03 M salts and (C) NaCl of increasing concentration. In this and following figures, the vertical deflections superimposed on the potential records represent the electrotonic potentials produced by a series of hyperpolarizing current pulses injected into the cell. Each millivolt of this deflection = 10 MΩ of system resistance (electrode + membrane input resistance) or cell membrane resistance alone for (C) only. Note resistance increase during hyperpolarization and decrease during depolarization. In all sections, horizontal bars above or below potential records indicate time of stimulus application.

Membrane resistances of SE cells decreased during depolarizations to concentrated salt solutions (except MgCl₂) and increased during the hyperpolarizations caused by more dilute solutions proportionally to the response amplitude (Fig. 3). KCl consistently caused a greater resistance change than NaCl at a particular response amplitude. In contrast to the other salts, the large depolarization evoked by 0.1 M MgCl₂ was associated with a several-fold increase in membrane resistance that, like the membrane potential, slowly (in tens of seconds) returned to the resting level after stimulation (Fig. 5). A smaller resistance increase was
caused by 0.03 M MgCl₂ regardless of the polarity of the associated potential change.

The distinguishing characteristic of the responses of TB-1 cells was a depolarization to K salts, even at 0.05 M (Fig. 6). At 0.1 M K salts often evoked large depolarizations of 40 or 50 mV which rose in a few seconds but decayed much slower after stimulation. These large responses were either maintained or partially phasic with a quick peak and a decrease of several millivolts to a maintained plateau. TB-1 cells gave responses of either polarity to 0.01 M K salts depending upon the resting potential at the time of stimulation. A cell could hyperpolarize to 0.01 M KCl at one time and depolarize later at a spontaneously lower resting potential. All TB-1 cells hyperpolarized to 0.003 M K salts and water. KCl, K₂SO₄, and KI were approximately equal in effectiveness, and at 0.1 M all three would depolarize the cell to the same potential level, regardless of membrane potential prior to stimulation (Fig. 7).

TB-1 cells responded to NaCl quite variably, some responding much like SE cells whereas others gave almost no response even to 1.0 M NaCl or H₂O. When present, the TB-1 cell responses to salt solutions (other than K salts) were similar to those of SE cells in polarity and form. Most stimuli, including MgCl₂, caused no or relatively small changes in the TB-1 cell membrane resistance. However, there was a consistent decrease in resistance with the large depolarizations to 0.1 or 0.3 M K salts.

A hyperpolarization to 0.1 M MgCl₂ solution was characteristic of TB-2 cells,
making them easy to distinguish from the other two types of cells. These responses had a slow time-course, especially after stimulation when the membrane potential slowly and irregularly returned to near the resting level (Fig. 5). In some cases, the cell remained hyperpolarized, which altered the subsequent responses to this or other stimuli. The membrane potential changes of TB-2 cells to salts other than MgCl₂ were indistinguishable from those of SE cells, though the associated conductance changes were absent, very small, or in the opposite direction.

![Comparison of responses of various 0.1 M salts in the three types of cells.](image)

**FIGURE 5.** Comparison of responses of various 0.1 M salts in the three types of cells.

![Responses recorded simultaneously from (A) a taste bud (TB-1) cell and (B) an epithelial (SE) cell to KCl solutions of increasing concentrations (molar).](image)

**FIGURE 6.** Responses recorded simultaneously from (A) a taste bud (TB-1) cell and (B) an epithelial (SE) cell to KCl solutions of increasing concentrations (molar).

**Responses of Lingual Cells to the Other Basic Taste Qualities**

Acid solutions (HCl and H₂SO₄ equally), even at 0.0003 M, evoked large depolarizations (e.g., 30 mV) and manifold increases in membrane resistance in SE cells (Fig. 8 B). Although the rate of rise of this depolarization was often rapid (e.g., < 200 ms to near peak depolarization), it was delayed by several seconds and occasionally was preceded by a small hyperpolarization. Both the membrane potential and resistance returned to the resting level extremely
slowly, taking as much as several minutes to recover from even a brief (e.g., 5-s) stimulus. More concentrated acid solutions decreased the delay and the rise times of the responses. The magnitude of this response was increased only slightly by increase in concentration, but the resistance change was increased significantly.

The depolarizing response of TB-1 cells to acid stimuli was highly variable from cell to cell and within the same cell at different times. Some of this variability, especially with a particular cell, appeared to be due to the change in the response at different membrane potential levels. When present, these depolarizations were quick to rise but slow to return to base line with little, if any, change in membrane resistance.

In TB-2 cells, acid solutions primarily evoked a biphasic response consisting of an initial brief depolarization immediately followed by a hyperpolarization that gradually but consistently decayed toward the resting membrane potential (Fig. 8). After the stimulus, the TB-2 cell would depolarize to or beyond the initial resting level, and in most cases, it would return to the resting potential within 1 min. Increasing the concentration of the acid solution increased the magnitude of the hyperpolarization. TB-2 cell membrane resistance remained mostly unchanged during this response.

Only 10 SE cells responded to 0.1 and 0.3 M sucrose or fructose solutions with a small (a few millivolts) depolarization or (n = 1) hyperpolarization. When they occurred, these responses were moderately slow to develop and showed no measurable resistance change. The most likely time to find a sugar response was shortly after penetration of an SE cell, suggesting that this response may be labile because of preparation decay or effects of other stimuli. The responsiveness of TB-1 cells to sucrose depended upon the membrane potential. When present, the response usually was a small depolarization with a slight increase in membrane resistance. Solutions of 0.3 M sucrose or fructose initiated biphasic responses in TB-2 cells very similar to those for acid, though with a slightly slower time-course. Membrane resistance changes were negligible with sweet stimuli. Fig. 8 C shows examples of sucrose responses.

Quinine (QHCl or QSO4) produced variable membrane potential changes in SE cells. The lower concentration (0.003 M) caused slow potential changes of either polarity with a slow return to resting potential. A 0.03 M solution usually evoked a slow but larger depolarization followed by a prolonged repolarization.

![Figure 7](image-url)
requiring tens of seconds. A characteristic effect of quinine on the SE cell was an enormous and progressive increase in membrane resistance to many times the resting level, proportional to concentration, which began at the stimulus onset regardless of the potential change. After stimulation, the resistance gradually decreased, frequently with an associated slow post-stimulus hyperpolarization of the cell before the return to the resting potential. Rather small depolarizations were evoked by QHCl solutions in some TB-1 cells (Fig. 8), though membrane potential level again seemed to be of importance. In general, quinine did not evoke a response in TB-2 cells, though a few slow, small hyperpolarizations were observed. Membrane resistance of both TB-1 and TB-2 cells changed little to QHCl.

Comparison of Responses in the Three Types of Cells

There were striking differences in the way the three types of cells responded to some of the stimuli employed. In this section, we compare responses which emphasize cell differences. Table I and the response profiles for cells within
each group (Fig. 9) summarize the major differences for the three types of lingual cells.

The best single stimulus for categorizing cells into types was 0.1 M MgCl₂. TB-2 cells primarily hyperpolarized to 0.1 M MgCl₂; whereas SE and TB-1 cells were depolarized by this stimulus, but an increase in membrane resistance occurred only in the SE cells. TB-1 cells were readily identifiable by their large and rapid depolarizations to K-salt solutions above 0.01 M, though their responses to other salts were smaller and variable in size and occurrence. Except for MgCl₂, all salts tested evoked rather similar responses in SE and TB-2 cells, including K salts.

In some cases, both SE and TB-1 cells depolarized to acid solutions, but the enormous resistance increase of SE cells distinguished the responses of the two cells. TB-2 cells gave an acid response of the opposite polarity without a change of membrane resistance. If present, SE and TB-1 cell responses to sugar solutions were small depolarizations, whereas TB-2 cells mostly hyperpolarized to sugar as well as to MgCl₂ and acid solutions. Like acid, quinine always increased the membrane resistance of SE cells, regardless of the potential change. This contrasted sharply with the lack of resistance change of TB-1 and TB-2 cells to quinine and acid stimuli.

Earlier experiments where lingual circulation was not carefully maintained revealed that SE cells continued to respond for > 1 h to most stimuli independent of blood supply to the tongue. However, responses from the two

### Table I

|                        | SE                          | TB-1                        | TB-2                        |
|------------------------|-----------------------------|-----------------------------|-----------------------------|
| Mean resting potential, mV | -20.1±1.1                   | -39.4±1.6                   | -36.2±2.0                   |
| Resting potential range, mV | -6 to -40                   | -20 to -64                  | -22 to -60                  |
| Mean input membrane resistance, MΩ | 26.5±2.7                   | 28.6±2.7                   | 23.6±2.9                   |
| Input membrane resistance range, MΩ | 10 to 70                   | 15 to 60                   | 15 to 50                   |
| Specific membrane resistance | 8,300 ohm cm²               | >8,300 ohm cm²              | >8,300 ohm cm²              |
| Effectiveness series | MgCl₂ >> NH₄Cl >> K₂SO₄ = aKCl >> MgCl₂ = NH₄Cl = Na₂SO₄ = K₂SO₄ | Variable                     |                             |
| Electrical coupling | +                           | -                           | +                           |
| Membrane resistance change (salts) | MgCl₂ +; rest -            | K salts -; rest 0           | All salts 0                 |
| Membrane resistance change (HCl) | +++                         | 0                           | 0                           |
| Membrane resistance change (acids) | + +                         | 0                           | 0                           |
| Circulation | Not necessary               | Necessary                   | Necessary                   |

+, increase; -, decrease; 0, no change.
FIGURE 9. Response profiles of 12 SE cells, 8 TB-1 cells, and 6 TB-2 cells to various stimuli. Depolarizations and hyperpolarizations are represented by positive and negative values, respectively; × indicates cell was not tested for that stimulus. Cells are arranged by amplitude of response to 0.3 M NaCl for SE cells, 0.1 M KCl for TB-1 cells, and 0.1 M MgCl₂ for TB-2 cells because of the significance of these stimuli (see text).
types of taste bud cells were only obtained when lingual circulation was maintained, thus indicating a fundamental difference between taste bud and non-taste bud cell physiology.

Possibly other cell types, perhaps less accessible for intracellular recording, exist within the tongue epithelium. Also, these categories might be further subdivided by more extensive testing. Unresponsive cells or cells responding like SE cells could be present in the taste buds along with the TB-1 and TB-2 cells.

**Effects of the Adapting Solution**

Inasmuch as the adapting 0.1 M NaCl solution is a sapid substance that may affect the parameters and responses of the cells, several experiments were conducted using less concentrated adapting fluids. Adapting SE and TB-1 cells to 0.01 M NaCl yielded slightly higher resting potentials and altered the magnitude of responses to NaCl solutions, but the polarity of the response still reversed at the new concentration of the adapting solution. The same order of effectiveness of salts was maintained for SE cells. When adapted to amphibian Ringer saline, the responses of SE cells were basically the same as under 0.1 M NaCl adaptation.

Switching to a water rinse after 0.1 M NaCl increased the resting potential of one TB-1 cell by ~19 mV. After adapting to the water, this cell depolarized 16 mV to 0.1 M NaCl, but the depolarization to 0.1 M KCl was nearly twice as large (30 mV). This proves that TB-1 cells actually were more sensitive to KCl than to NaCl solutions and that the larger responses to K-salt solutions were not merely due to a reduction of sensitivity to NaCl by adaptation.

**Electrotonic Coupling between Lingual Cells**

A small degree of electrotonic coupling was found for many, though not all, pairs of adjacent SE cells (Fig. 10 A). Although coupling tended to be weak with coupling ratios (voltage change in follower cell/voltage change in current-inject cell) averaging ~ 0.1 and not exceeding 0.3, it was consistent (19 pairs of cells) and large enough to make the possibility of recording artifacts unlikely. Coupling usually decreased within minutes after penetration in most cases, possibly due to the inherent difficulties of simultaneous recordings from adjacent cells and membrane damage caused by the electrodes. Also, coupling was more easily demonstrated at the beginning of an experiment, suggesting that these intercellular connections could be disrupted with time.

Spread of the injected Procion navy blue dye into one or more of the neighboring epithelial cells was frequently observed in situ and gave additional evidence for intercellular connections. Bennett (1973) proposed that channels connect coupled cells at gap junctions and permit passage of current-carrying ions and dye molecules. Dye spread and electrotonic coupling were found for both deep and surface epithelial cells (Fig. 11 B). TB-2 cells had coupling ratios similar to those for SE cells (e.g., 0.11 for cells in Fig. 10 C). In all four instances of simultaneous recordings from two neighboring TB-2 cells within a bud, some coupling was observed. Coupling did not change significantly when it was monitored by repeated current pulses for several minutes in one pair of TB-2
cells during chemical stimulation. Electrically coupled pairs, whether SE or TB-2 cells, always gave very similar responses to chemical stimuli. Small amounts of current passed well in either direction between coupled cells of both types indicating nonrectifying electronic synapses.

Recordings from seven pairs of TB-1 cells failed to show any coupling (Fig. 10 B), suggesting, but not proving, that TB-1 cells are not coupled. No coupling was found between cells of different types, but it was never shown that these cells were truly neighboring.

As controls, large current pulses were passed with one electrode outside a cell while recording intracellularly with the other yielding no indication of coupling. In Fig. 10 A, the electronic potential changes in the follower cell (V-2) decreased and disappeared as the electrode left the cell, as evidenced by the loss of recorded membrane potential. In addition, injection of dye into both cells of a coupled pair was observed in a number of preparations, though not confirmed histologically.

Identification of Recording Site by Dye-Marking

Injection of dye into a cell after recording served as a reliable indicator that the electrode tip was actually inside a cell and allowed location of the cell histologi-
Figure 11. Histological identification of recording site in (A) surface and (B) deep epithelial cells by injected Procion navy blue dye. Dye was localized near the nucleus of an SE cell (A, indicated by arrow) but spread to two cells in B.
Intracellular Responses of Mudpuppy Lingual Cells

When intracellular dye injection was successful, the dye was clearly localized in one or more cells and did not diffuse away rapidly upon rinsing. Most of the cells in this report gave an in situ indication of dye injection, though successful histological verification was obtained for only 16 of them.

Because of the loose adhesion of the surface cells in the mudpuppy tongue, the SE cells frequently would detach from the epithelium when the electrode was withdrawn, making clear histological localization rare. One example is shown in Fig. 11 A, where the dye was localized in the cell around the nucleus. Deep epithelial cells, which gave small or no response to chemical stimulation, were more readily retained and subsequently identified within the tissue. Fig 11 B shows dye localized in two deep cells, suggesting dye spread between electronically coupled cells.

Dye was injected in five TB-1 and three TB-2 cells and subsequently identified in section. Examples of dye-injected TB-1 and TB-2 cells shown in Fig. 12 A and B, respectively, reveal cells unmistakably within taste buds. Note the underlying dermal papillae that cup the oval-shaped clusters of elongated cells. In Fig. 12 B two cells appear to contain dye, which might indicate dye crossing between a pair of coupled TB-2 cells.

**DISCUSSION**

*Factors Affecting Cellular Characteristics*

Previously reported resting potentials of toad and frog lingual cells adapted to Ringer solution (containing 111 mM NaCl) are close to the mean resting potential of -20.1 mV for mudpuppy SE cells adapted to 0.1 M NaCl. The higher values for frog, rat, and hamster taste cells adapted to more dilute solutions are consistent with the observations reported here.

The NS (NaCl-sensitive) and WS (water-sensitive) cells of the frog, which gave responses of opposite polarity to water or NaCl solutions (Sato and Beidler, 1973), are the only taste cells that have been categorized previously on the basis of their responses. Previous studies may not have tested the cells in a way that allowed classification. For example, WS cells were not found by Akaike et al. (1976) when the frog tongue was adapted to 0.01 M NaCl instead of the Ringer saline used in the previous study, implying that the adapting solution employed is an important factor for differentiating cells.

Also, the responsiveness of a cell to a particular taste quality may vary, depending upon such factors as the effects of previous stimuli, modulation from centrifugal regulation, or lateral interactions. These factors could change the membrane potential of the taste cell (İsakov and Byzov, 1971; Kutyna and Bernard, 1977) and thus alter subsequent responses. TB-1 cell membrane potential level at the time of stimulation greatly affected its responses, especially to non-salt stimuli; therefore, a TB-1 cell could have quite different response profiles at different membrane potentials. The responsiveness of TB-1 cells was independent for the four taste qualities so that the degree of sensitivity for the
Figure 12. Histological identification of dye-injected (A) TB-1 and (B) TB-2 cells. Note dye in A (indicated by arrow) is near cell nucleus in base of bud and in B is in two cells near periphery of bud.
different qualities varied among TB-1 cells whereas the overall sensitivity of each cell depended upon the resting potential level.

Responses of Non-Taste Bud Cells

Eyzaguirre et al. (1972) recorded responses to chemical stimulation from surface cells on the toad tongue regardless of their location with respect to the taste discs. The SE cells of the mudpuppy also gave responses to sapid solutions, many of which resembled taste cell responses in this and other species. Though non-taste bud cells generally are not believed to play a role in taste reception, the large responses from SE cells (e.g., to acid solutions) show that they have the capability of being involved in taste, perhaps in a simpler, less discriminative manner than taste bud cells. A potential change generated in a large number of SE cells simultaneously might generate sufficient field-potentials around the taste buds to affect their input to the intragemmal nerve fibers. Such an action in the toad was suggested by Eyzaguirre et al. (1972) for surface cells that surround rod cell processes in the taste disc.

In the mudpuppy lateral line organ, the responses to NaCl and KCl solutions recorded intracellularly by Yanagisawa et al. (1974) were quite similar to the SE cell responses to these stimuli. This is particularly significant for taste, since Katsuki (1973) found that the lateral line organs in a number of adequate vertebrates responded to chemical stimuli like primitive organs of taste.

Response Mechanisms

Both Akaike et al. (1976) for frog cells and Ozeki (1971) for rat cells proposed that the taste cell depolarization produced by NaCl or KCl was caused by an increase in membrane ionic permeability, possibly to Na or Cl. Such a mechanism might be involved in SE cell responses to these and other salts. However, the membrane resistance increase with depolarization to MgCl₂ indicates that this stimulus acts by a different mechanism, possibly by a decrease in resting permeability to K⁺, as proposed by Krnjevic et al. (1976) to explain the depolarization with increased membrane resistance that was observed upon injection of Mg²⁺ into cat spinal motorneurons.

A decrease in membrane permeability to K⁺ also was suggested as the mechanism of depolarization and increased membrane resistance to QHCl (Ozeki, 1971; Akaike et al., 1976). Akaike and Sato (1975) proposed that bitter substances act by penetrating the taste cell membrane and dislocating its crystal lattice, thus altering permeability. Quinine solutions probably increase SE cell membrane resistance by this same mechanism. Though acid solutions may act by the same mechanism, it is possible that low pH depolarizes SE cells by decreasing membrane permeability to Cl⁻. Fink and Lütgau (1973) found that the permeability of striated muscle cell membrane to Cl⁻ decreased in low pH solutions, and Brown (1972) demonstrated that low pH affected a neuronal membrane potential by changing Cl⁻ permeability.

In some cases the response mechanism differed among the cell types. In particular, MgCl₂, H₂SO₄, and QHCl must have depolarized SE and TB-1 cells by different mechanisms, because membrane resistance changes were different.
The hyperpolarizing responses of TB-2 cells to MgCl₂, acid, and sugar solutions were associated with very small changes in membrane resistance. One explanation might be that large depolarizations in closely neighboring cells (TB-1 or SE cells) cause a passive potential change of the opposite polarity across the TB-2 cell membrane. Nolte and Brown (1972) suggested that tightly packed cells in the median ocellus of Limulus could be hyperpolarized temporarily by depolarizing currents generated across adjacent photoreceptor cell membranes. The initial small depolarization preceding these responses and the potential changes to other salts could be the direct response of the TB-2 cell itself that was early or large enough to override the effects of currents from other cells.

Function of Electronic Coupling in Tongue Epithelium

The occurrence of electrotonic coupling in the lingual mucosa of the mudpuppy is not surprising inasmuch as it has been demonstrated for every epithelial tissue that has been tested (see Loewenstein, 1973). There are several possible functions for coupling in this tissue. An entire sheet of SE cells all responding in concert to a change in bathing solution could produce sufficient current, especially at the "holes" in this sheet created by the taste pores (or pits), to affect the cell membrane potentials within the taste pores where taste transduction classically is presumed to take place. If SE cell potential changes affect taste reception, transfer of potentials from cell to cell because of coupling could mediate a positive feedback between cells which synchronizes and enhances their responses. Loewenstein et al. (1965) stated that even the small group of coupled cells in a sensory epithelium could act as a signal amplifier. Assuming that coupled TB-2 cells have a direct effect on taste reception, similar amplification or integration of responses may occur. The decrease of input resistance in TB-2 cells seen with large hyperpolarizing current pulses (Fig. 2) may be due to increased coupling similar to the restoration of coupling by cell repolarization reported for salivary gland cells (Rose and Loewenstein, 1971).

For most epithelial cells, a more likely function of coupling is for metabolic, nutritive, or regulatory interactions. This possibility for SE and TB-2 cells does not exclude an electrical role for the observed communications.

Taste Bud Cell Structure and Function

From the results of cell dye-marking in this study, we propose the hypothesis that our physiologically differentiated TB-1 and TB-2 cells correspond to the morphologically differentiated light and dark cells, respectively, for the following reasons: (a) Farbman and Yonkers (1971) reported that light cell nuclei tend to lie deeper and more centrally within the mudpuppy taste bud, and dyed TB-1 cell nuclei are similarly located, as a whole. (b) Likewise dark and TB-2 cells both have nuclei more toward the periphery of the bud. (c) It was easier to locate, penetrate, and record for longer periods from TB-1 cells than from TB-2 cells which could be due to the more voluminous, regular shape reported for light cells. (d) Inasmuch as dark cells surround and largely separate light cells, the larger areas of contact between dark cells would provide greater opportunity for junctions mediating electrotonic coupling as observed between TB-2 cells.

Many responses of the TB-2 (putative dark) cells resembled the hyperpolar-
izing secretory potentials recorded from cat sublingual gland (Lundberg, 1956). This suggests that TB-2 cells may have a secretory function, as proposed by Farbman and Yonkers (1971) and Murray (1971) for dark cells. They also proposed a supportive role for dark cells, and electrotonic coupling between supportive elements (TB-2 cells) could be for nutritive supply, as suggested for coupled glial cells. However, the large responses of TB-1 cells, especially to K salts, implies a chemoreceptive function, either alone or in concert with the TB-2 cells, thus providing additional input for finely differentiating between taste stimuli.

The authors wish to thank Dr. John Teeter for helpful criticism, Miss Pat McCarty for histology, Miss Carol Dizack for graphics, and Mr. T. P. Stewart for photography. This work was submitted in partial fulfillment of the requirements for the Ph.D. degree to Michigan State University by the senior author and was supported in part by grant NS-09108 from the National Institutes of Health.

Received for publication 27 December 1977.

REFERENCES

AKAIKE, N., A. NOMA, and M. SATO. 1973. Frog taste cell response to chemical stimuli. Proc. Jpn. Acad. 49:464-469.

AKAIKE, N., A. NOMA, and M. SATO. 1976. Electrical responses of frog taste cells to chemical stimuli. J. Physiol. (Lond.). 254:87-107.

AKAIKE, N., and M. SATO. 1975. Effects of local anesthetics on frog taste cell responses. Jpn. J. Physiol. 25:585-597.

AKAIKE, N., and M. SATO. 1976 a. Mechanism of action of some bitter-tasting compounds on frog taste cells. Jpn. J. Physiol. 26:29-40.

AKAIKE, N., and M. SATO. 1976 b. Water response in frog taste cells. Comp. Biochem. Physiol. A Comp. Physiol. 54A:149-156.

BENNET, M. V. L. 1973. Permeability and structure of electrotonic junctions and intercellular movements of tracers. In Intracellular Staining and Neurobiology. S. B. Kater and C. Nicholson, editors. Springer-Verlag New York, Inc. 115-134.

BROWN, A. M. 1972. Effects of CO2 and pH on neuronal membranes. Fed. Proc. 31:1399-1403.

ESAKOV, A. I., and A. L. BYZOV. 1971. Electrical response of the frog’s gustatory cells to centrifugal stimulation. Bull. Exp. Biol. Med. (Eng. trans. Byull. Ekp. Biol. Med.). New York Consultant Bureau, New York. 72:723-726.

EYZAGUIRRE, C., S. FIDONE, and P. ZAPATA. 1972. Membrane potentials recorded from the mucosa of the toad’s tongue during chemical stimulation. J. Physiol. (Lond.). 221:515-532.

FARBMAN, A. I., and J. D. YONKERS. 1971. Fine structure of the taste bud in the mumpupu, Necturus maculosus. Am. J. Anat. 151:353-370.

FINK, R., and H. C. LÜTTGAU. 1973. The effect of metabolic poisons upon the membrane resistance of striated muscle fibers. J. Physiol. (Lond.). 234:29-30P.

KATSUKI, Y. 1973. The ionic receptive mechanism in the acoustico-lateralis system. In Basic Mechanisms in Hearing. A. R. Möller, editor. Academic Press, New York. 307-334.

KIMURA, K., and L. M. BEIDLER, 1961. Microelectrode study of taste receptors of rat and hamster. J. Cell. Comp. Physiol. 58:131-140.
KRNJEVIC, K., E. PUIL, and R. WERMAN. 1976. Intracellular Mg ++ increases neuronal excitability. Can. J. Physiol. Pharmacol. 54:73–77.

KUTYNA, F., and R. A. BERNARD. 1977. Effects of antidromic activity in gustatory nerve fibers on taste disc cells of the frog tongue. J. Comp. Physiol. A. Sens. Neural. Behav. Physiol. 118:291–306.

LOEWENSTEIN, W. R. 1973. Membrane junctions in growth and differentiation. Fed. Proc. 32:60–64.

LOEWENSTEIN, W. R., S. J. SOCOLAR, S. HIGASHINO, Y. KANNO, and N. DAVIDSON. 1965. Intracellular communication: renal, urinary bladder, sensory, and salivary gland cells. Science (Wash. D. C.). 149:295–298.

LUNDBERG, A. 1956. Secretory potentials and secretion in the sublingual gland of the cat. Nature (Lond.). 177:1080–1081.

MURRAY, R. G. 1971. Ultrastructure of taste receptors. In Handbook of Sensory Physiology. Chemical Senses 2: Taste. L. M. Beidler, editor. Springer-Verlag New York, Inc. 4:31–50.

NOLTE, J., and J. E. BROWN. 1972. Electrophysiological properties of cells in the median ocellus of Limulus. J. Gen. Physiol. 59:291–306.

OZEKI, M. 1970. Hetero-electrogenesis of the gustatory cell membrane in rat. Nature (Lond.). 228:868–869.

OZEKI, M. 1971. Conductance change associated with receptor potentials of gustatory cells in rat. J. Gen. Physiol. 58:688–699.

OZEKI, M., and M. SATO. 1972. Responses of gustatory cells in the tongue of rat to stimuli representing four taste qualities. Comp. Biochem. Physiol. A. Comp. Physiol. 41A:391–407.

ROSE, B., and W. R. LOEWENSTEIN. 1971. Junctional membrane permeability. Depression by substitution of Li for extracellular Na, and by longterm lack of Ca and Mg; restoration by cell repolarization. J. Membr. Biol. 5:20–50.

SATTO, T. 1969. The response of frog taste cells (Rana nigromaculata and Rana catesbeana). Experientia (Basel). 25:709–710.

SATTO, T. 1972. Multiple sensitivity of single taste cells of the frog tongue to four basic taste stimuli. J. Cell. Physiol. 80:207–218.

SATTO T. 1976. Does an initial phasic response exist in the receptor potential of taste cells? Experientia (Basel). 32:1426–1428.

SATTO, T., and L. M. BEIDLER. 1973. Relation between receptor potential and resistance change in the frog taste cells. Brain Res. 53:455–457.

SATTO, T., and L. M. BEIDLER. 1973. Membrane resistance change of the frog taste cells in response to water and NaCl. J. Gen. Physiol. 66:735–763.

STENSAAS, L. J. 1971. The fine structure of fungiform papillae and epithelium of the tongue of a South American toad, Calyptocephalella gayi. Am. J. Anat. 131:443–462.

TATEDA, H., and L. M. BEIDLER. 1964. The receptor potential of the taste cell of the rat. J. Gen. Physiol. 47:479–486.

YANAGISAWA, K., V. TAGLIETTI, and Y. KATSUKI. 1974. Responses to chemical stimuli in the hair cells of the lateral-line organ of mudpuppy. Proc. Jpn. Acad. 50:526–531.