INTRACELLULAR CHARACTERISTICS AND RESPONSES TO GUSTATORY STIMULATION OF CELLS IN THE MUDPUPPY TONGUE

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Rudy A. Bernard
Major professor

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ABSTRACT

INTRACELLULAR CHARACTERISTICS AND RESPONSES
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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 in SE cells, and $MgCl_2$, acids and quinine greatly increased the membrane resistance. One type of taste bud cell (TB-1) was characterized by large depolarizations to K-salt stimulation, and the other type of taste bud cell (TB-2) characteristically hyperpolarized to $MgCl_2$, acid and sugar solutions. Both TB-1 and TB-2 cell responses were accompanied by membrane resistance changes that were relatively small compared to those of SE cells.

Electrotonic coupling was observed for pairs of SE and TB-2 cells but not for pairs of TB-1 cells nor cells of

different types. Dye marking of cells after recording their responses allowed verification of results *in situ* and histologically. From the identification of cells in section, it is hypothesized that 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 functions of electrotonic coupling are discussed in relation to taste reception.

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By
Charles H. Hutchison Keesor West

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This work is dedicated to my parents who have unendingly offered their faith, encouragement, assistance and love; and to my wife for her constant support, loyalty, devotion and love.

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LITERATURE REVIEW

Introduction

Classically, the chemical solutions that elicite taste sensations are divided into the four basic taste qualities of salt, sour, sweet and bitter. When investigating the electrophysiology of taste, responses to chemical stimulation usually are evaluated according to the quality of the stimulus.

Taste information is carried to the CNS of vertebrates along nerve fibers in cranial nerves VII, IX, and X. Recordings from whole nerves, especially the chorda tympani branch of VII and the glossopharyngeal (IX) in mammals, have revealed that different species have different orders of sensitivity for various chemical stimuli. For example, even comparing two salts, rodents respond more to NaCl than to KCl, while carnivores show a greater response to KCl. Most single taste fibers respond by differing degrees to a variety of stimuli from more than one quality. This lack of single fiber specificity might arise, at least in part, from the innervation by each fiber of several taste organs.

Primary taste reception is assumed to occur within the taste buds or discs, clusters of specialized epithelial cells contacting both nerve fibers and the fluid medium. Membrane potential changes, or receptor potentials, recorded from taste bud cells are thought to be the result of physical interactions of taste ions or molecules with receptor molecules in the membrane of that cell. By some chemical or electrical means of transmission, the taste cell activity alters the firing rate of the innervating nerve fiber. Like the single fibers, taste bud cells are multiply sensitive to taste qualities, though occasionally there is some correlations between responses to different qualities. Lateral interactions and centrifugal regulation at the periphery influence the taste input carried centrally by the nerve fibers (see reviews by Sato, 1971, 1973).

Many questions concerning taste reception by the cells remain unanswered, including receptor mechanisms, taste cell differentiation, interaction mechanisms, and correlation between cell structure and function. The following study investigates these questions.

Intracellular Recording from Taste Receptors

Electrical Properties of Taste Cells

Recordings from inside gustatory cells were first reported by Kimura and Beidler (1956,1961) from taste bud

cells in rats and hamsters. They recorded abrupt negative shifts of 30 to 50 mV in the DC potential upon thrusting a glass pipette microelectrode into the tips of fungiform papillae, which contain the taste bud. Similar potential shifts, assumed to be the resting potentials of penetrated cells, have been recorded since then in a number of studies on the tongue of rats, frogs and toads.

In general, resting potential values for lingual cells were relatively low, and they varied with the different species and adapting solutions used (see Table 1). Another dimension to intracellular taste recordings was the input resistance of the taste cell membrane as measured with small pulses of current passed through the recording electrode by means of a bridge circuit. Reported input resistance values were quite variable, even in different studies on the same species (Table 1).

Ozeki (1971) extended the investigation of taste cell electrical parameters in rats. The electrotonic potentials produced across rat taste cell membranes by square current pulses had a decay that was a simple exponential function, in most cases, with a time constant of 15.5 msec. By using an approximation of the cell membrane area and the input resistance, the specific membrane resistance for these cells was calculated as 536 ohm cm^2. Dividing the time constant by the specific resistance yielded a specific membrane capacity of 28.9 $\mu F/cm^2$.

Table 1. Taste Cell Electrical Characteristics

Study	R.P. (-mV)	R_M (MΩ)	Species	Rinse
Kimura & Beidler (1956,1961)	30-50		R, H	water
Tateda & Beidler (1964)	30-50		R	water
Ozeki (1970,1971)	40.1	81.2	R	0.0414 M
Ozeki & Sato (1972)	40.1	81.2		NaCl
Eyzaguirre et al. (1972)	17.6		T	Ringer
Sato (1969,1972)	24		F	Ringer
Sato & Greenberg (1972)	10-40	39	F	Ringer
Sato & Beidler (1973)	10-40			
Kutyna (1973)	10.5		F	Ringer
Sato & Beidler (1975)	17.8(NS)	39.6(NS)	F	Ringer
Sato & Beidler (1975)	19.2(WS)	32.0(WS)		
Esakov & Byzov (1971)	20-40		F	--
Akaike et al. (1973)	30		F	0.01 M
Akaike & Sato (1975,1976a,b)	30			NaCl
Akaike et al. (1976)	42.8	17.3	F	0.01 M NaCl

R = rat; H = hamster; T = toad; F = frog; R.P. = resting potential; R_M = input membrane resistance; NS = NaCl sensitive; WS = water sensitive cell.

A plot of injected current and resultant membrane potential change in rat taste cells gave a linear relationship for depolarizations and hyperpolarizations down to -30 mV below the resting potential; however, greater hyperpolarizations revealed a non-linear relationship (Ozeki, 1971). A similar current-voltage plot revealed a linear relationship and no rectification over a wide range of membrane potentials for frog taste cell membranes (Sato and Greenberg, 1972). These findings and the absence of action potentials suggest that taste bud cells present no regenerative responses nor any rectification (Sato, 1973).

Receptor Potentials and Membrane Conductance Changes

Every intracellular study to date has shown that most cells believed to be taste bud cells respond to a stimulus solution above a particular critical concentration with a slow depolarization that is largely maintained during stimulation. This membrane potential change is thought to be the receptor potential. This potential has a magnitude and time course that is dependent upon the stimulus concentration and rate of application, stimulus quality, and various other factors. Associated with this receptor potential, there usually is some change in membrane conductance, as measured by repeated application (1/sec) of brief hyperpolarizing current pulses. The characteristics of these responses

vary among the cells (even within a taste organ) and among the species studied. Individual gustatory cells show multiple sensitivities to the four basic taste qualities, in most cases responding to three or four of the qualities (see review by Sato, 1973).

Kimura and Beidler (1956,1961) reported receptor potentials to NaCl concentrations above 0.005 M, which increased in magnitude with increasing stimulus concentration up to a saturation level usually between 0.5 and 1.0 M. Although each cell had different sensitivities, a plot of integrated responses of ten cells against stimulus concentration was quite similar to one for chorda tympani nerve responses. Also, the NaCl function basically fit the equation describing taste reception as adsorption of stimulating ions or molecules to independent receptor sites on the taste cell membrane (Beidler, 1954).

Testing the depolarizing effectiveness of various 0.1 M salts, Kimura and Beidler (1961) found a sequence of NaCl>KCl>NH_4Cl with $CaCl_2$ and $MgCl_2$ falling either higher than NaCl or variably lower among the other stimuli. A series of Na-salts with various anions supported the hypothesis that the cation primarily has an excitatory effect and the anion acts as an inhibitor. When testing solutions from each of the four qualities, the largest response generally was to 0.01 M HCl followed by 0.1 M Nacl,

0.02 M quinine hydrochloride (QHCl), with 0.5 M sucrose being least effective. Even in hamsters where there was a large neural response to sucrose, the cellular response to 1.0 M sucrose was relatively small (1-6.5 mV).

A later study of rat taste buds revealed that the resting potentials and responses to stimuli varied as the electrode was lowered through the bud, passing through cells of different sensitivities. Near the bottom of the taste bud, probably with the electrode tip outside but next to a cell, the response would be small and of reversed polarity (Tateda and Beidler, 1964).

In these earlier studies of rat taste cells, the tongue was rinsed with water following each application of a test solution. However, Ozeki (1970,1971; Ozeki and Sato, 1972), studied the same receptors, with 0.0414 M NaCl (an equivalent concentration to rat saliva) as a rinse and adapting solution. Overall, the responses to solutions representing the four taste qualities were similar to those of the previous studies; however, one difference was a small hyperpolarizing response to NaCl solutions less concentrated than the adapting solution.

In addition, membrane conductance was monitored during chemical stimulation by the technique of injected short pulses of equal current. Depolarizations produced by NaCl, KCl, HCl and sucrose were accompanied by increased

conductance of the receptor cell membrane; whereas, QHCl produced depolarizations with a decrease in membrane conductance. These differences in membrane conductance during receptor depolarizations were interpreted to imply a different mechanism of reception for quinine compared to the other stimuli.

In studying adaptation, Ozeki (1971) found that rat taste cells showed greater adaptation to more concentrated NaCl stimuli. During adaptation, membrane conductance changed very little and was slow to return to the resting level following simulation. This fact suggested that the process of adaptation cannot be attributed to membrane conductance change alone. With the other taste qualities, the sucrose response adapted faster than that for NaCl; and the responses to HCl and QHCl adapted more slowly (Ozeki, 1971; Ozeki and Sato, 1972).

In a later report on rat taste bud cells, Ozeki and Sato (1972) stated that with a stimulus flow rate of 1.2 ml/min, the depolarizing responses to most stimuli reached a maximum in about five seconds and fell to the resting potential in about the same time course after stimulation. However, responses to 0.01 M HCl had a much slower rise (40-50 sec) and fall time. Though the rise time for KCl responses was nearly the same as for NaCl, the decay after a high concentration of KCl was long (40 sec or more).

The cells usually were more sensitive to NaCl than to KCl. In general, the magnitude and rate of rise of a receptor potential increased with increasing stimulus concentration.

Thresholds for a depolarizing response to solutions representing the four taste qualities, with the tongue adapted to 0.0414 M NaCl, were as follows: 0.05-0.1 M NaCl; 0.001-0.005 M QHCl; 0.001-0.005 M HCl; 0.01-0.05 M sucrose (Ozeki and Sato, 1972; Sato and Ozeki, 1972). These thresholds were in fair agreement with the chorda tympani data, although the receptor threshold for NaCl was slightly higher than the threshold for the nerve, probably due to adaptation of the cells to saline solution rather than water.

Receptor potentials recorded in frog taste cells have many of the same characteristics as those of the rat. The responses were mostly sustained slow depolarizations (time to peak averaged 7-8 sec for all qualities) with the magnitudes related positively to the stimulus concentration; most cells were sensitive to more than two taste qualities, though specific sensitivities varied from cell to cell even within the same taste disc (Sato, 1969,1972a,b).

When changes in membrane conductance and potential of frog taste cells were recorded to water and NaCl solutions of various concentrations, two types of cells were distinguished (Sato and Greenberg, 1972; Sato and Beidler, 1973, 1975). The NS (NaCl-sensitive) cells depolarized to NaCl

solutions more concentrated than 0.1 M and hyperpolarized to more dilute NaCl solutions and water. The WS (water sensitive) cells generally gave smaller responses of the opposite polarity; but in both types of cells, depolarizations and hyperpolarizations were associated with proportional increases and decreases in membrane conductance respectively.

In a recent study, Akaike and Sato (1976b) found only NS cells since they all depolarized to NaCl solutions more concentrated than the adapting solution (Ringer or 0.01 M NaCl) and hyperpolarized to more dilute solutions or water. They proposed that the well-known large water response recorded in frog glossopharyngeal nerve is generated by the spontaneous reduction of the initially large water-induced hyperpolarization seen in the taste cells.

A change in membrane conductance of frog taste cells was associated with the receptor potentials, though its magnitude and direction depended upon the stimulus. The response profiles and conductance changes for many cells were similar for groups of stimuli, suggesting similar receptor mechanisms for the members of a group. These groups were NaCl and KCl, $CaCl_2$ and $MgCl_2$, sucrose and glucose, various acids (hydrochloric, acetic, lactic and tartaric), and various bitter substances (QHCl, brucine and caffeine). The cells had variable sensitivities for different groups

and for the various stimuli within a group, though most cells responded to all groups except sucrose and glucose. An increase in membrane conductance was associated with most responses (depolarizations); exceptions were the depolarization to bitter substances and the hyperpolarization to dilute NaCl and KCl solutions, both accompanied by decreases in membrane conductance. Threshold values and stimulus-response curves approximately agreed with those determined for the IXth nerve response (Akaike et al., 1973, 1976; Akaike and Sato, 1976a; Sato and Akaike, 1975).

Sato (1969, 1972a,b) obtained qualitatively similar responses to the various taste qualities even though he used an isolated frog tongue adapted to Ringer saline. In his preparation, receptor potentials had latencies of 100-300 msec or possibly less. Also, Sato (1972a) showed that the receptor potentials for sucrose and quinine during prolonged stimulation decreased (adapted) faster than those for NaCl and acetic acid.

When Eyzaguirre et al. (1972) studied the surface cells of isolated toad lingual mucosa, all cells penetrated, regardless of location, responded to sapid substances. The various salts tested (NaCl, KCl, NaF, $CaCl_2$, $MgCl_2$ and Na_2SO_4) produced similar slowly rising depolarizations; however, responses to $MgCl_2$ and $CaCl_2$ were larger and slower to decay, whereas Na_2SO_4 was generally less effective.

As with most frog taste cells, water evoked a hyperpolarization, but here, repeated applications showed summation of the responses. The three acids tested (HCl, H_2SO_4 and H_2NO_3) yielded variable or biphasic responses. Under proper conditions, neither sucrose nor QHCl yielded a receptor potential. Quinine was effective in inhibiting the NaCl response, and the current-voltage plot indicated that QHCl increased the membrane resistance as reported by Ozeki (1970,1971) and others for taste cells.

Distribution of Sensitivities in Taste Cells

Since individual taste cells were shown to be sensitive to more than one taste quality, tests were made to determine whether or not the responses to different stimuli occurred independently. For frog cells, Sato (1969,1972a) reported no correlation between any stimulus pair, and he indicated that the sensitivities to the different qualities were distributed randomly and independently on the taste cells. Sensitivities in single taste fibers were not random, however, since there was an excessive proportion (30%) of narrowly sensitive fibers indicating some selectivity in the connections between fibers and cells (Sato, 1972a). In another study of frog gustatory cells, the only significant correlation between stimulus pairs was for NaCl and HCl (Akaike et al., 1976).

As in the frog, rat taste cell sensitivities were distributed randomly and independently. However, there was a slight negative correlation for NaCl and sucrose and some positive correlation for NaCl with HCl and QHCl. After making consideration for differences in adapting solutions, the proportion of elements responding out of the total tested was about the same for taste cells and fibers in the rat (Ozeki and Sato, 1972; Sato and Ozeki, 1972).

Factors Affecting Gustatory Cell Properties

Inherent in any investigation of effects of chemical solutions on a cell are the influences the adapting solution has on the properties of that cell. This fact is illustrated by comparing the intracellular taste reports when different adapting or rinse solutions were used. For example, frog taste cells had mean resting potentials of -35 mV when adapted to 0.01 M NaCl and -24.1 mV when adapted to Ringer saline (Akaike et al., 1976; Akaike and Sato, 1976b). Also, the responses of frog taste cells and nerves were smaller when the adapting solution was Ringer rather than 0.01 M NaCl (Akaike and Sato, 1976a; Akaike et al., 1976). The concentration at which NaCl responses in frog taste cells reverse polarity depends, in part, on the adapting solution concentration (Akaike and Sato, 1976b).

Ozeki and Sato (1972) reported an augmentation of HCl responses and a slight suppression of sucrose responses of rat gustatory cells when test solutions were made in 0.0414 M NaCl instead of pure water. Since a similar potentiation of the responses to HCl had been seen in cat chorda tympani fibers by prior adaptation of the tongue to NaCl (Wang and Bernard, 1969), the difference between cellular and nerve response in rats could be due to the different adapting solutions used (Ozeki and Sato, 1972).

An adapting solution of 0.01 M NaCl and 0.001 M QHCl depressed frog taste nerve and cell responses to various stimuli and reduced the cell resting membrane potential and conductance (Akaike and Sato, 1976a). Similarly, QHCl depressed NaCl responses in toad lingual cells, but alone it did not evoke a response in these cells after it was buffered (Eyzaguirre et al., 1972).

Several other results from toad cells indicated the influence on responses by other sapid substances applied concurrently or previously to the cells. Hyperpolarization of the toad cells by previous water stimulation enhanced the depolarization to NaCl solutions. Prolonged exposure to certain acids (HCl, H_2SO_4) had a deleterious effect on the preparation and its responsiveness. Also, both $MgCl_2$ and $CaCl_2$ had slowly decaying responses, and occasionally the membrane potential failed to return to its resting

level following stimulation by 0.5 M $CaCl_2$ (Eyzaguirre et al., 1972).

The rate of rise of the receptor potential is proportional to the rate of stimulus solution flow, as demonstrated in frog taste cells (Akaike et al., 1976). Sato and Beidler (1975) suggested that the lack in taste cell responses of the transient initial peak seen in the whole nerve recordings might be due to the slower flow rates used in intracellular recording to avoid dislodging the microelectrode. In the mudpuppy, the magnitude of the single fiber response was directly proportional to the rate of stimulus onset, and the peak transient response disappeared, in general, with stimulus flow rates below 30 ml/min (Samanen, personal communication).

Several studies have revealed the effects of various pharmacological agents on taste cell physiology. Effects varied for the different agents tested, some being totally ineffective whereas others had drastic effects. For example, cocaine depolarized rat taste cells and reversed the polarity of their responses to NaCl (Tateda and Beidler, 1964). In the same species, neither procaine nor tetrodotoxin had a significant effect on the characteristics of taste fibers or cells (Ozeki and Noma, 1972).

In a study of the effects of local anesthetics on the frog tongue, procaine and lidocaine evoked a small depolarization and increased membrane resistance in taste cells

that was seen in the glossopharyngeal nerve as a transient response. Prolonged application of these anesthetics to the tongue depressed nerve response maximally to QHCl, less to salt, and least to acid. The same order of effectiveness was seen for their depressant action on receptor potentials in taste cells. These lipid soluble anesthetics may act by penetrating the cell membrane and dislocating the membrane structure so that the ionic conductance (perhaps to K^+) is depressed. The similarities to the effects of quinine suggests that it might act by the same mechanism as these anesthetics (Akaike and Sato, 1975).

It has been established by a number of reports that centrifugally conducted activity in the IXth nerve of the frog affects the taste responses from nerve and receptor cells. Esakov and Byzov (1971) demonstrated that electrical stimulation of the glossopharyngeal nerve initiated a hyperpolarization of the frog taste cells that was proportional to the frequency of stimulation. When recording from the tongue surface, this response to nerve stimulation was seen as a positive wave that reportedly came from the surface epithelium of the whole tongue. They recorded intracellular action potentials in response to nerve stimulation that were unrelated to the application of taste stimuli. These spikes were believed to have been recorded from the synaptic thickenings histologically observed in

taste discs, and these results were thought to indicate a naturally occurring centrifugal regulation of frog taste cell activity.

Kutyna (1973) also recorded a hyperpolarization in frog taste cells in response to IXth nerve stimulation as well as a simultaneous depolarization from the surface cells of the taste disc (primarily) and the surrounding epithelium. Depending on stimulus conditions, the electrical stimulation of the nerve could produce depression or enhancement of the nerve response to taste stimulation. The finding of similar cell potential changes due to electrical stimulation of neighboring fungiform papillae supports the hypothesis that lateral interaction occurs between neighboring taste organs through such a mechanism.

Chemosensory Responses of Other Systems

Several sensory systems other than taste are known to respond to chemical stimulation, and intracellular recordings have been obtained in three such systems. Olfactory cells differ from taste bud cells in that they are primary afferent neurons as well as being the receptors, though both systems are chemical senses. Recordings have been made from olfactory cell somata as well as from their axons, revealing changes in membrane potential and spike activity associated with application of stimuli (Aoki and Takagi,

1968; Gesteland and Farbman, 1973). The carotid body is a chemo-sensitive structure primarily consisting of unmyelinated nerve terminals and associated (glomus) cells, thus resembling taste bud structure. Glomus cells are responsive to changes in K^+ concentration and pH (like taste cells); however, it is uncertain whether glomus cells or other elements (e.g., nerve endings) are the receptors in the carotid body (Eyzaguirre et al., 1975).

The responses of the lateral line system are of particular importance to taste for two reasons: 1) the structure of the neuromast organ strongly resembles taste bud structure and 2) the lateral line cells have been shown to be highly sensitive to chemical changes in their fluid environment. For these reasons, the effects of chemical solutions on lateral line organs are described here.

When Sand (1975) used the mudpuppy lateral line organ for a study of the effects of changing ionic environments on their mechano-sensitivity, he found that the responses to vibrations were proportional to the Ca^{++} concentration of the external medium. In solutions made Ca^{++}-free with chelating agents, the organ did not respond to vibrations; and it was postulated that the depolarizing receptor current of the hair cells was carried primarily by Ca^{++}. K^+ and Na^+ were equally effective in enhancing the mechano-sensitivity of these organs, but they were less effective than Ca^{++}.

Suppression of sensitivity by La^{++}, Co^{++} and Mg^{++} appeared to be due to a competitive blocking of Ca^{++}. Both the resting firing rate and the mechano-sensitivity were reduced by solutions with low pH (Sand, 1975).

Intracellular recording of the responses to stimulation by monovalent salts of the free neuromasts of the mudpuppy was reported by Yanagisawa et al. (1974). Resting potentials averaged -42 mV for hair cells adapted to 0.115 M NaCl (isotonic NaCl solution). Solutions of NaCl or KCl more concentrated than the adapting solution evoked depolarizations in these cells with concurrent increased membrane conductance; whereas, less concentrated solutions and distilled water evoked hyperpolarizations and decreased membrane conductance. The magnitude of the membrane potential and conductance changes of these hair cells was proportional to the difference in concentration between the adapting and test solutions.

By plotting the stimulus concentration/receptor potential with concentration, a straight line relationship was obtained for KCl and NaCl. This indicated that these results from hair cells agree with Beidler's taste equation for a mono-molecular reaction between the taste receptor site and the stimulus. Since the nerve discharges from neuromasts also fit this same linear equation, the receptor potentials linearly affected the nerve discharge rate.

These results suggest that the mudpuppy lateral line organs may serve as chemoreceptors, at least to simple salts, as well as mechanoreceptors (Yanagisawa et al., 1974).

Katsuki et al. (1970) demonstrated the chemosensitivity of the pit organs (free neuromasts) of several species of sharks as revealed by change in lateral line nerve discharge rate. Monovalent cations were excitatory in the following order of effectiveness: K^+, $Rb^+ > Na^+$, $NH_4^+ > Cs^+$, Li^+. Of the divalent cations tested, Ca^{++} was the most inhibitory with Mg^{++} and Sr^{++} less inhibitory. Ba^{++} seemed to have an irreversible toxic effect on the organ.

Anions had only a slight effect on the response, suggesting that the primary excitant was the cation, as in taste buds of other animals. The other taste qualities (acid, sugar and quinine) were weakly or variably effective; therefore, these chemoreceptors on the body surface appeared more primitive and simple than those of the oral cavity though they were highly sensitive to salts. Due to the action of pharmacological inhibitors, the authors concluded that the hair cells of the neuromasts were the actual receptors and not the nerve endings. Also, the salt solutions enhanced the nerve response to electrical and mechanical stimulation of these organs (Katsuki et al., 1970).

The lateral line nerve of teleosts also was responsive to chemical stimuli, and this nerve showed similar responses

in marine fish and sharks. Both fresh water and marine fish gave greater responses to KCl than to NaCl though this difference was exaggerated in the marine fish due to the high Na^+ content in their environment. Catfish were quite unique for the following reasons: divalent cations were stimulating rather than suppressive; some fibers responded much more to sodium glutamate than to NaCl; there were ammonium specific fibers; some fibers responded to quinine though none responded to sugar solutions. Terminal buds that resemble taste buds were found in the flank skin of catfish; and because of their source of innervation, it was concluded that these were the taste organs of the skin. Although several species of teleosts have the terminal buds, many fish (e.g., the mullet) lack them but have free neuromasts that respond to monovalent cations (Katsuki, 1973).

Lateral line organs of the amphibian, Xenopus laevis, were responsive to mono- and divalent cations, but Na^+ had peculiar effects. Na^+ was much less stimulating than K^+ or even the glutamate anion, and Na^+ was slightly suppressive at very low concentrations. This animal and another amphibian, the bullfrog, lose their tails with its lateral line organs along with their skin chemical sensitivity during metamorphosis, and they subsequently develop their taste organs and sense within the oral cavity (Katsuki, 1973).

Considering these and the other results, the lateral line organ may be regarded as a primitive taste organ; and due to the enhancement of mechano-sensitivity by K^+, it also may serve as a model for the inner ear of higher animals. Cyclostomes, elasmobranchs, teleosts and amphibian tadpoles all have lateral line organs that are similarly responsive (e.g., to monovalent cations); the comparable end-organs in adult amphibians and catfish appear to have greater and more developed chemical sensitivity (Katsuki, 1973).

Histology of Taste Buds

Numerous studies with light and electron microscopy have investigated the vertebrate taste buds (or discs), the structures assumed to be the end-organ for taste (see reviews by Graziadei, 1969; Murray, 1971; Murray and Murray, 1971). In general, taste buds may be described as groups of modified epithelial elements that are clustered into a barrel-shaped aggregate extending from the underlying connective tissue up to the surface of the mucosa. Usually a pit (containing a homogeneous acidophilic substance) is formed by the specialized apical ends of the taste bud cells at the surface. Nerve fibers enter the bud from the underlying tissue and ramify within the bud making contact with

most or all of the bud cells. At least two types of fusiform-shaped cells, one considered sensory and the other primarily supportive, form the bud in addition to the basal cells in the lower portion of the structure (Murray and Murray, 1970).

Taste Bud Cell Types

The presence of different cell types within the taste bud is fairly well accepted and universal in all vertebrates studied; however, there is some disagreement on function and origin of these cellular types. Classically, the mature cells were divided into dark and light cells, the dark cells being the receptor elements, because of their apical processes and resemblance to other sensory cells, and the light cells being the supportive or sustentacular elements for the maintenance of the receptive cells. Careful studies of the ultrastructure of taste buds of different tongue areas from several species of mammals by Murray and co-workers have led them to disagree on the function of the cell types and to distinguish a third mature cell type.

From their work in rabbit foliate taste buds, which are fairly typical of most vertebrate buds, they believe that the type I (dark) cells are the supporting elements for three main reasons: 1) they appear to secrete the substance filling the taste pit, 2) they surround the nerve fibers and other cells similar to glia cells in nervous

tissue, and 3) they appear to act as phagocytic cells in normal and degenerating taste buds. The less numerous type II (light) cells also contact chemical stimuli through the pore and have an intimate relationship with the nerve fibers, though the "typical" synaptic structure was not observed. However, signals still may be generated in these adjacent nerves since unspecialized contact points have been found in other receptors. Due to this apparent connection between stimulus and nerve, the type II cells are believed to be primary receptor cells of taste. Unlike the other cell types, the third type of taste bud cell, the type III cell, has the classic synaptic arrangement. Their penetration of the pit substance and their synapses with the nerve fibers make the type III cells another candidate as a gustatory receptor (Murray, 1971; Murray and Murray, 1970, 1971).

The last type of cell in the taste bud, the basal cell, is found in the lateral basal margin of the bud not contacting the surface. Ever since it was shown that taste bud cells are constantly being renewed (e.g., Beidler and Smallman, 1965), many investigators suggested that the different types of taste bud cells are actually different stages in the life cycle of a single type of cell (Murray, 1971). Contrary to this hypothesis, several lines of evidence have shown that, in rabbit foliate taste buds, each of the three mature cell types arises independently,

probably from basal cells; and each category of cell has a turnover (Murray and Murray, 1971).

Studies of other mammalian and non-mammalian taste buds have revealed a basic structure quite similar to the rabbit taste bud, though some specific features do vary. Morphological indications of both afferent and efferent synapses between taste bud cells and innervating nerve fibers have been seen in many vertebrates. This reciprocal synapse may be involved in efferent regulation, a feedback mechanism, or trophic influences on taste bud cells (Graziadei, 1974).

The taste organs of frogs and toads are unique in that they form a disc-shaped plaque of cells that covers the upper surface of the fungiform papillae. The two types of cells that contact the surface are called the associate cell, assumed by some to have no sensory function, and the sensory, rod, or receptor cell, thought to be the primary receptor because it has distal processes contacting the surface and its proximal poles make typical chemical synapses with the nerve fibers at the base of the organ (Graziadei and De Han, 1971; Murray, 1971; Stensaas, 1971).

Fine Structure of Mudpuppy Taste Buds

Farbman and Yonkers (1971) studied the fine structure of the taste buds of Necturus maculosus, because the exceptionally large size of their cells makes them likely

candidates for electrophysiological studies. The taste buds of Necturus are almost twice the length and twice the diameter of those of other vertebrate species studied (100-150 μ long by 90-120 μ wide). The exceptional size is due to both larger cells and greater number (80-100) of cells in each bud.

In general, the form of the bud is much like that found in most other vertebrates, containing closely packed, elongated cells and unmyelinated nerve processes in the lower third of the bud. One difference from mammalian taste buds is the lack of a taste pore or pit, since the apical ends of the cells form an exposed round area on the surface about 20-30 μ in diameter.

The typical cell categories of dark (60%), light (30%), and basal (10%) cells were observed. As in other species, the most numerous dark cells (type I cell of Murray) form the periphery of the bud and are intermixed with the light cells centrally. Their large, oval nuclei were usually found higher in the bud than those of the light cells. Clusters of membrane-bound granules separated by bundles of filaments were found in the apical cytoplasm of the dark cells, which had highly irregular shapes with many branching processes that seemed to partially insulate the light cells and ensheath the nerve processes. Like the dark cells of mammalian taste buds, the morphology of these dark cells suggests a secretory function.

The light cells also had large, oval nuclei, but they tended to be located in the central, proximal part of the bud. Characteristically, the light cell cytoplasm contained exceptionally large amounts of agranular endoplasmic reticulum and many mitochondria, resembling the chloride cell in fish gill epithelium and other cells involved in ion transport. If the endoplasmic reticulum of light cells is like that of chloride cells, it would be continuous with the external membrane, thus representing an expanded membrane system. Ionic fluxes across the membrane system may be the source for the changes in membrane potential of these cells. The authors considered the morphology of the light cell to indicate a gustatory receptive function. This hypothesis was supported by the finding that nerve endings are adjacent to light cell profiles containing 100 nm vesicles, suggestive of an afferent synapse.

Basal cells were located next to the basal lamina in the lower portion of the bud. Unique to basal cells were dense core vesicles 70-90 nm in diameter, frequently located opposite the basement lamina. These vesicles resemble the dark-core vesicles of rabbit type III cells and the vesicles in neurons believed to contain catecholamines.

The unmyelinated nerves entering the bud from the subgemmal plexus rarely were found above the nuclear level of the cells, and they varied in diameter from 0.5-2.0 μ.

Although no "typical" synapses were found, occasionally 100 nm vesicles were seen in light cells opposite an expanded nerve process. The tight junctions found just beneath the surface of the bud might permit electrical coupling between cells, as suggested in different species by others.

Electrotonic Coupling

During the last 20 years, there have been numerous discoveries of an intimate type of intercellular communication that is now thought to be vitally important for many cellular interactions in embryonic and adult tissues. This cell-to-cell communication, known as electrotonic coupling, has been demonstrated in a wide variety of tissues including excitable and non-excitable tissues in many animal species throughout the animal kingdom. A phenomenon that is so universal as to be found in cells as diverse as those of sponges and mammalian brain must be of some functional importance. Yet, its functions must be different for such dissimilar tissues as the segments of the lateral giant axon of crayfish and the salivary gland cells of fly larvae. Although electrotonic coupling may have different purposes and locations, there are some general features of this process that are similar for most cases (see reviews by Bennett, 1973a; Loewenstein, 1970, 1973; Staehelin, 1974).

Evidence for Intercellular Communication

The first known exceptions to the membrane prototype as a continuous barrier effectively separating each cell were found by Weidman in 1952 in cardiac muscle fibers and by Furshpan and Potter in 1957 in certain synapses of the crayfish nerve cord. In these and in most studies showing electrotonic coupling since then, the method of measurement involved multiple intracellular electrodes for recording and/or passing current. Due to the inherent difficulties of this technique, the tissues tested have been limited to those with large cells; and coupling has been found in most of them (see Loewenstein, 1970; Satir and Gilula, 1973).

For the passage of current-carrying ions and larger molecules between cells, there must be actual physical contact at a specialized junction. Several types of cellular junctions occur, but it is generally accepted that the structural basis for electrotonic coupling is the gap junction (Staehelin, 1974). Unlike the true tight junction, the gap junction has a 20-40 Å intermembrane space forming a "gap" between the cells. Tracers (e.g., lanthanum) have been used to penetrate this gap and reveal a hexagonal lattice of subunits. Externally applied tracers and freeze-fracture studies also reveal cylinders and particles aligned with depressions suggesting small intercellular channels bridging the gap that connect the cytoplasms of the coupled cells (Satir and Gilula, 1973; Bennett, 1973b).

There is a close correlation between the incidence of gap junctions and the degree of coupling. For example, treatments that left only gap junctions in the intercalated discs between cardiac muscle cells left their coupling intact. Disrupting these gap junctions abolished the electrical coupling as well (Staehelin, 1974).

A key point of functional concern in the structure of gap junctions is the cytoplasmic bridges crossing the gap. One proposed structure that fits both morphological and physiological studies consists of two sets of channels. One set, the extracellular channels, runs parallel to the gap, possibly allowing movement of materials in the extracellular space; the other set, the intercellular channels, connects the two cells with bridges that restrict diffusion of ions or molecules depending on the number and size of the channels (most about 10 Å diameter) (Bennett, 1973b).

The injected current used to measure electrotonic coupling is carried through the junction as ions, probably K^+, Na^+ and Cl^-. In the heart, K^+ is known to pass freely through the tissue at the junctions; and labelled Na, K, Cl, Co, I and SO_4 ions as well as labelled sucrose all cross the septum in the septate axon of the crayfish. To test the size and degree of permeability of the channels, molecules larger than the small ions were used.

Fluorescent or colored dyes were injected into one cell of a coupled pair to give a visual indication of whether or not they cross the junction. In the crayfish septate axon, fluorescein (M.W. 332), neutral red (M.W. 252), and Procion yellow (M.W. 630) all crossed the junctions. Liver cells in culture, as well as in vivo were shown to be coupled by the junctional spread of fluorescein, dansyl-L-glutamate, dansyl-DL-asparate and Procion yellow. In the skate retina, the horizontal cells were coupled and passed Procion yellow. Loewenstein and co-workers found that molecules with molecular weights as high as 69,000 (bovine serum albumin) would pass between dipteran salivary gland cells, including the dyes fluorescein and Procion yellow. Fluorescein mixed with peroxidase solutions revealed crossing in the septate axon of microperoxidase (M.W. 1800) but not horse radish peroxidase (M.W. 40,000), though the passage of the microperoxidase may be post-fixation artifact (Bennett, 1973b; Staehelin, 1974).

Other systems that are electrically coupled, especially in embryos, fail to pass dyes. For example, fluorescein does not pass between pairs of coupled blastomere cells of the teleost, Fundulus; nor does it or Procion yellow pass between coupled echinoderm embryonic cells. In the Xenopus embryo, fluorescein failed to cross in the early blastula stages, although it did cross in reaggregated cells from

later stages (Bennett, 1973b). The tracer experiments showing failure of dye to cross out-to or in-from extracellular spaces support the hypothesis of a sealed channel between coupled cells. These channels are selective, possibly due to difference in the diameters of the hydrophilic channels (Bennett, 1973b).

For excitable cells, one obvious function of low-resistance junctions is for electrotonic transmission between cells; however, for non-excitable tissues, a more elusive function of coupling may be related to the demonstrated interchange of ions and molecules. This opens the possibility that the function of these junctions is to permit the passage of such substances as nutrients, metabolites, and regulatory molecules (Staehelin, 1974).

The exchange of metabolites and nutrients would have an equalizing or buffering effect on the tissue tending to distribute work loads on cells. For example, glia with end feet on capillaries could supply coupled neighbors with needed nutrients. In the squid embryo, the loss of coupling between yolk cells and other cells occurs soon after the onset of circulation. The more restrictive junctions in other embryos may be designed to allow selective passage of small cations or molecules that can influence differentiation and other cell processes (Bennett, 1973a; Staehelin, 1974).

The theoretical "optimal" size for morphogens, molecules that may critically alter cells, is 300-500 M.W., which is in the same size range as fluorescein and Procion yellow. Though not all cancerous cells lack the coupling that is found in normal cells, tumor cells from mammalian liver, thyroid, and stomach epithelium all fail to show normal communication. Using a culture of hepatoma cells, it was possible to show a parallel between electrotonic and metabolic coupling and presence of gap junctions in hybrids of normal and malignant cells. Furthermore, labeled metabolic precursors injected into one of the coupled pair of Retzius cells in the leech CNS were shown to cross into its partner cell, presumably through the site of coupling (Rieske et al., 1975). These studies and others support the idea that growth-controlling molecules may pass through gap junctions (Loewenstein, 1970, 1973; Staehelin, 1974).

Coupling in Epithelial and Sensory Systems

Loewenstein and his associates have studied coupling in a variety of tissues, especially in epithelial systems. They have come to the conclusion that intercellular communication is a rather general phenomenon in cell association and most cells in a given tissue usually are interconnected (Loewenstein, 1966, 1973).

They found coupling in every type of epithelium that was studied, including the following from several species: cells of salivary gland, liver, kidney, thyroid, skin, urinary bladder, stomach, gut, and sensory epithelia (Loewenstein, 1970). The entire cell system was interconnected in salivary gland, renal tubules, and liver cells; whereas, communication was restricted to strings of cells in urinary bladder and small clusters of cells in a sensory epithelium. This coupling of numerous cells allows the entire system to constitute a unit, with elements operating in concert. In the case of the sensory epithelium of elasmobranchs, the function of coupling between small (2-3) groups of cells was synchronization of excitation, making the connected system act as a signal amplifier (Loewenstein et al., 1965; Loewenstein, 1966).

Mammalian epithelial tissues in which electrotonic coupling has been demonstrated include the liver (Loewenstein and Penn, 1967), the thyroid (Jamakosmanovic and Loewenstein, 1968), the salivary glands, and the exocrine pancreas (Petersen and Ueda, 1976). All acinar cells within one pancreatic or salivary acinus were closely coupled, but there was no communication with different acini.

Two urodele amphibians (Ambystoma and Triturus) have intercellular ionic communication between adjacent

epidermal cells with an average coupling ratio of 0.4. Communication between epidermal cells was also found in frog skin (Loewenstein and Penn, 1967). Propagated electrical events involving coupling in surface epithelial tissues have been recorded from many invertebrates and certain vertebrate embryos and larvae (Spencer, 1974). Systems in Necturus that show intercellular communication include the renal tubules, gastric mucosa, and the gall bladder. All cells of the gallbladder were coupled (Frömter, 1972); however, in the gastric mucosa coupling was only between cells of the same kind (i.e., surface or oxyntic cells). Surface cell coupling was limited so that injected current spread along cell cables (Blum et al., 1971). Electrotonic coupling has also been found between invertebrate epithelial cells such as in molluscan gill (Gilula and Satir, 1971) and in epideraml sheets in beetle larvae (Caveney and Podgorski, 1975).

Perhaps the best known example of electrotonic coupling within a sensory system is for the lateral eye of Limulus. Each eye is composed of many ommatidia, each containing 12 retinular cells surrounding an eccentric cell. All the cells within an ommatidium are coupled with each other but not to cells in other ommatidia. Illumination causes a slow depolarization of the retinular cells that is electrically transmitted to the eccentric cell. An interesting observation is the loss of coupling with sufficient

hyperpolarization of a retinular cell (i.e., a rectifying synapse). Also, the degree of coupling is greater in the dark than in the light (Smith and Baumann, 1969).

Alteration of Junctional Membrane Permeability

Loewenstein and colleagues have proposed the hypothesis that the amount of membrane-bound Ca plays a key role in formation of the junctions that mediate electrotonic coupling. From a series of experiments on the salivary gland cells of Chironomus, they believe that junctional membrane permeability is inversely related to the amount of free Ca^{++} in the cytoplasm. Normally the Ca^{++} activity of the cytoplasm is low, but processes that raise this activity tend to uncouple cells. These processes include injection of Ca^{++} into the cell, allowing Ca^{++} to enter through a hole in the membrane, and inhibition of energy metabolism by poisons or cooling, thus inhibiting the normal energy-dependent removal of Ca^{++} (Loewenstein, 1973).

Uncoupling of cells was demonstrated in earlier experiments in salivary gland and liver cells by removel of Ca^{++} from the external medium (Nakas et al., 1966; Loewenstein, 1966). When external Ca^{++} is low, internal Ca^{++} rises due to increased influx or decreased efflux and uncoupling results. A depolarization of the cell accompanied this uncoupling, and repolarizing the cell restored junctional

conductance (Rose and Loewenstein, 1971). Other processes cause decoupling by allowing Ca^{++} (or Mg^{++}) to leak into the cell through some break in the membrane caused by trypsin digestion, anisotonicity, alkalinity or chelation (Loewenstein et al., 1967). Uncoupling may also be induced by allowing other divalent cations to enter the cell (Oliveira-Castro and Loewenstein, 1971). In the crayfish septate axon, uncoupling was caused by mechanical injury of the axon, which was associated with a separation of the junctional membranes (Asada and Bennett, 1971; Pappas et al., 1971).

STATEMENT OF PROBLEM

As described above, taste reception is believed to involve an interaction of stimulus molecules or ions and receptor sites on taste bud cell membranes, which initiates electrical changes in the membrane distant from the receptor site. These changes generate a receptor potential which in some manner initiates or alters the discharge of innervating nerve fibers (e.g., Sato and Beidler, 1975). Intracellular studies on taste organs have yielded data indicating that cells have wide-ranging sensitivities to taste stimuli, implying a specialized receptive role for these cells. However, Eyzaguirre et al. (1972) recorded similar responses from taste disc and non-taste disc surface cells, thus leaving the extent and function of taste organ specialization in question.

One objective of this study was to compare the responsiveness of taste bud and general (non-specialized) epithelial cells to sapid solutions within the same tongue. The mudpuppy tongue offered certain advantages for intracellular recording, since the large size of the cells and taste buds permits identification of the recording sites and confirmation of the validity of the recordings.

Lateral interactions of taste organs via branching peripheral nerve fibers have been described (e.g., Kutyna, 1973), and anatomical studies have suggested the possibility of electrotonic coupling in taste organs (Farbman and Yonkers, 1971; Stensaas, 1971). If coupling were extensive within taste buds or lingual epithelium, it could have a significant influence on taste reception and possibly provide another means for peripheral interactions. Another objective of this study was to test for the occurrence and extent of electrotonic coupling between lingual epithelial cells, both in and out of the taste buds.

Classically, taste bud cells are divided into two adult types, light and dark cells; their functions have been suggested from structural traits but not tested physiologically. It was hoped that these recording and marking experiments might give some indication of the functional role of the two mature cell types.

MATERIALS AND METHODS

Preparation

Experiments were performed using adult mudpuppies (Necturus maculosus) of 15 to 30 cm in length. Prior to use, these animals were maintained at low temperature (4 to 10° C) in tanks of filtered, aerated water. To avoid fungal infections, tetracycline (about 16 mg/liter) was added to the water when the tanks were cleaned every other week.

In earlier experiments, animals were anesthetized by submersion in a 5% solution of Urethane (ethyl carbamate) until movement of the gills ceased. However, this procedure had the disadvantage of restricting or eliminating lingual circulation. Later experiments revealed that maintained circulation to the tongue was not necessary for general epithelial cell responsiveness, but it was essential for obtaining responses from taste bud cells. To retain the vital lingual circulation, the mudpuppies were lightly anesthetized by a 1.5 to 2.5 ml I.P. injection of a 20% urethane solution, and the final level of anesthesia was carefully reached by slowly dripping the urethane solution

over the gills. Thus, the proper surgical plane with the necessary elimination of gill beating without impairment of circulation to the tongue was reached.

During the experiment, the animal was secured and kept moist in a plastic chamber with its mouth held wide open to allow access to the non-distendible tongue. Slightly tilting the bottom of the chamber to partially raise the animal's body above its head enhanced the blood flow through the tongue, which was periodically checked throughout the experiment. The animal was adapted to room temperature (23°) for more than an hour before recording was begun.

Adapting and Stimulating Solutions

Tongues were adapted to a constantly flowing solution of 0.1 M NaCl in most of the experiments. There were several reasons for using this as the adapting solution: 1) It allowed measurement of resting potentials from surface cells that would not be possible with a pure water rinse (i.e., a 0 potential could not be determined with the electrode tip in water). 2) Recording stability with the high-resistance electrodes was much greater with a saline rather than a water rinse, the baseline was steadier, and it was easier to record for long periods of time. 3) Although a Harris amphibian Ringer saline rinse had the same advantages mentioned above, it tended to cause cell shrinkage or

distortion of some of the surface cells with prolonged application; whereas, 0.1 M NaCl caused no apparent change in the surface epithelium even after several hours. 4) Harris Ringer solution rinse also had the advantage of approximating the extracellular fluid in the tongue, but it contained a mixture of salts that made it difficult to properly relate its adaptive effects on the cells to the stimulating actions of mono-salt solutions. This problem was avoided with 0.1 M NaCl. 5) This NaCl concentration was probably greater than that of the saliva or mucus normally covering the animal's tongue (e.g., 0.0414 M for rat saliva; see Ozeki, 1971) but lower than the NaCl concentration in an isotonic solution (0.115 M) for this species (Yanagisawa et al., 1974). Therefore, 0.1 M NaCl was probably a reasonable balance for the continuously flowing solution employed in this study.

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 Na_2SO_4; 0.01 - 0.3 M K_2SO_4; 0.03 - 0.3 M NH_4Cl; 0.01 and 0.1 M KI; 0.03 - 0.1 M $MgCl_2$. All salts, including the adapting NaCl, were dissolved in deionized water with resistivity greater than 2 megohms/cm. This same deionized water also served as a stimulus.

Other stimuli were 0.003 and 0.03 M QHCl and 0.003 M QSO_4; 0.0003, 0.001 and 0.003 M H_2SO_4 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.

Flow System

Since microelectrodes can be easily dislodged from cells by any mechanical disturbance, a system was designed that would allow a constant flow of solution over the tongue even when the solution was changed. This system permitted prolonged intracellular recordings with rapid exchange between adapting and stimulating solutions without mechanical artifacts. Total exchange of fluids and arrival of the stimulus at the recording site could be estimated to within one second.

Basically, the system consisted of two separate gravity-fed flow systems for the adapting and stimulating solutions, which were connected to a switching valve (Figure 1). One of the two outputs from this valve was connected by a fine polyethylene tube to a 3 mm diameter chamber directly over the recording site on the tongue, and the second output went to a waste container. When the valve was switched, connections between the two inputs and outputs

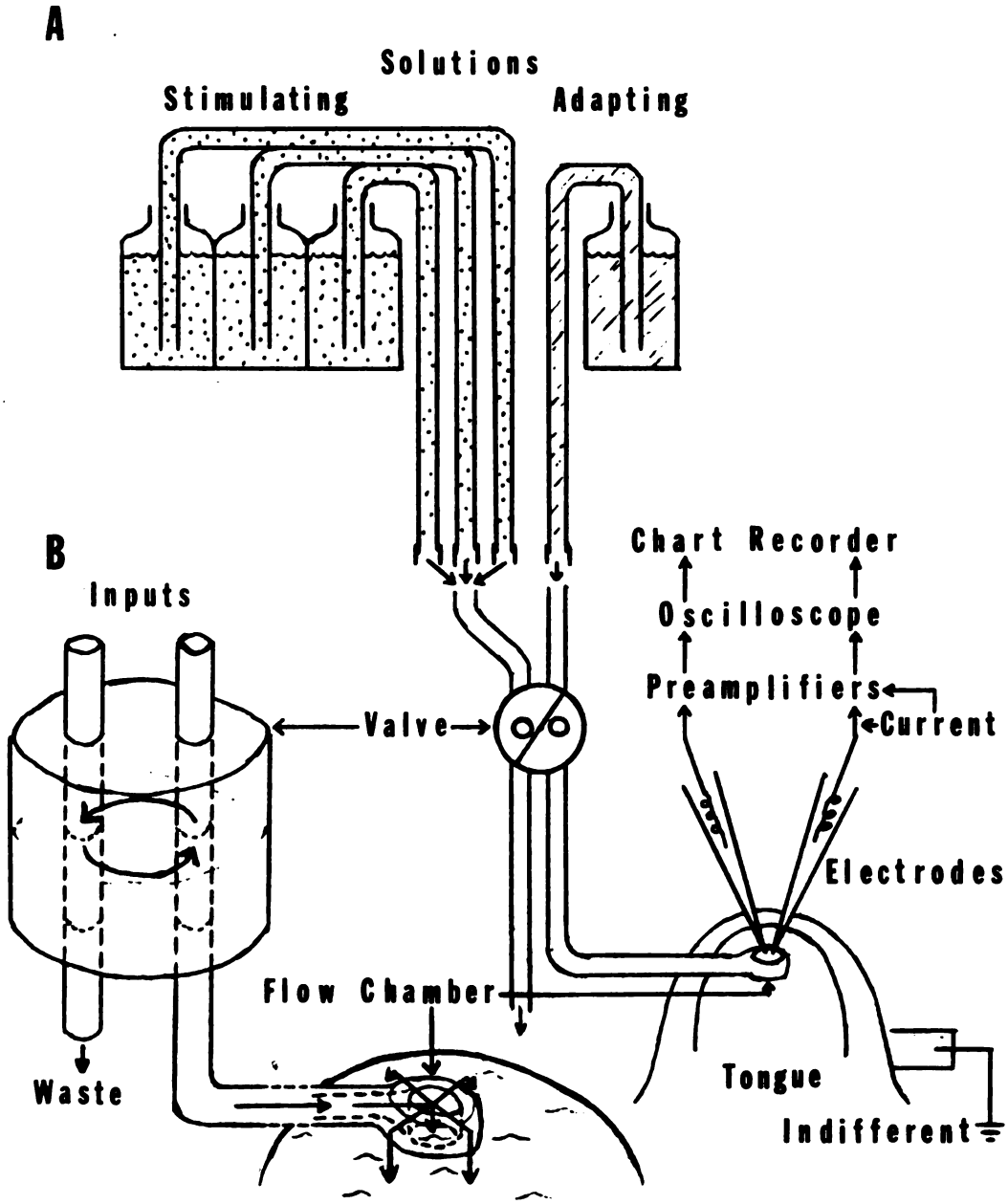

Figure 1. Schematic representation of the experimental set up. A, general scheme; B, simplified diagram of switching valve and flow chamber.

were reversed with minimal mixing of the two solutions, thus very rapidly changing from adapting to stimulating condition. To stop stimulating, the valve was switched to its original position; and if desired, a different stimulating solution could be attached to the valve. This system provided a flow rate of about 4.6 ml/min.

The chamber on the tongue allowed fluid to enter from the side and to exit over the open top, then flowing down off the tongue to a waste container. This simple flow chamber formed a small pool of adapting or stimulating solution over an area of the tongue that could be reached by microelectrodes lowered through the pool.

Electrodes

Glass pipette microelectrodes were pulled from acid cleaned 1 mm O.D. capillary tubing containing a fine glass fiber fused to the inner wall (Frederick Haer and Co.). As confirmed with scanning electron microscopy, these electrodes had outer tip diameters of less than 0.5 μ, and their DC resistance, determined by 1 nA current pulses, ranged from 60 to 100 megohms when filled with a Procion dye solution (see below). When filled with 2.7 M KCl, the electrode resistance was about half that for the dye-filled electrodes. Electrodes with initial resistances greater

than 100 megohms or tip potentials greater than -30 mV were not used.

The fiber fused inside the electrode made it possible to back-fill the electrodes by inserting a fine (31 gauge) needle into the stem end. Solutions that were used to fill the electrodes were injected from a syringe through a Swinney filter apparatus, thus allowing ultrafiltration of the solution as it was injected into the electrode.

Most of the microelectrodes were filled with a Procion dye solution containing 10% dye with 3% sodium bicarbonate (alkaline pH allowing higher dye concentration) made in deionized, distilled water. The most frequently used dye was a Procion navy blue H3R (ICI America, Inc.) that gave relatively low resistance, stable electrodes and the best injection results. Other dye solutions were tried on occasion, especially a mixture of Procion rubine MX-B and Procion black HN (Polysciences, Inc.). In a few experiments, electrodes were filled with 2.7 M KCl, which yielded essentially the same experimental results as the dye-filled electrodes. The electrodes were connected to preamplifier probes by holders containing a Ag-AgCl half cell (WPI) filled with 2.7 M KCl.

The indifferent electrode was a short (one inch) piece of 5 mm polyethylene tubing containing Ringer agar. This tube was slipped partially over another KCl-filled electrode

holder, and the agar at the other end of the tube was placed in contact with the moist skin on the animal's head.

Recording Apparatus and Resistance Measurement

Outputs from the recording electrodes were led into two high impedance, capacity compensated DC preamps (WPI models M4-A and 750). From these, the signals went to a dual beam storage oscilloscope (Tektronix 5103N) for display and to a polygraph (Gilson ICT-5H with IC-MP amplifiers) for a permanent record.

Current could be injected while recording by means of a bridge circuit in the model M4-A. A constant monitor of system resistance (i.e., electrode plus cell membrane resistance) was achieved in this system by injecting 0.1 nA constant current pulses through the electrode at the rate of 1 pulse/sec. By Ohm's law, a potential deflection of 1 mV corresponded to a resistance of 10 megohms. In most recordings, the electrode resistance was not balanced in the bridge circuit so that cell membrane resistance was determined as the difference between system resistances before and after penetration. A few experiments (e.g., coupling tests) were conducted with the electrode resistance balanced out, thus recording only electrotonic potential changes across the cell membrane resistance.

Tests for electrotonic coupling were performed by impaling two adjacent cells and injecting current into one of the cells while recording the membrane potentials in both cells. If significant coupling were present, simultaneous electrotonic potential changes would be recorded from both cells. The degree of coupling is expressed as the coupling ratio: the voltage change in the follower cell/the voltage change in the injected cell.

Impalement of Cells

Unlike the papillae-covered tongues of other vertebrates, the tongue of the mudpuppy is rather smooth except for the slightly raised eminences that each contain a single taste bud (Farbman and Yonkers, 1971). Lateral illumination of the tongue and a single capillary loop that was seen at the base of each bud with good lingual circulation were helpful in locating the buds. In some cases, the taste buds themselves could be visualized within the somewhat translucent epithelium.

Once a bud was located, the flow chamber was oriented directly over that tongue area and an electrode was gradually advanced through the pool of adapting solution into the bud region. As the electrode tip entered the tissue, small, slow shifts in the measured resistance and potential preceded a larger, abrupt negative drop in the potential

that signaled cell penetration. Although all or part of this potential difference (i.e., the resting potential) decayed soon after penetration of some cells, only cells that retained most of this potential for more than a minute after penetration were subsequently used for chemical testing. An increase in the system resistance usually accompanied this penetration.

Intracellular recordings with fine, high resistance electrodes, as used in this study, are susceptible to physicochemical artifacts generated at the electrode tip, and changing the solution bathing the cells and the electrode (i.e., chemical testing) complicates this problem. To control for these artifacts, the following steps were taken: 1) Artifact potential and resistance changes evoked by test solutions were compared to the responses of the cells, revealing a number of significant differences. 2) Responses were reduced or absent in deep epithelial cells or decaying preparations. 3) Dye injected into cells after recording their responses was localized within the cells when seen in section. Further details are presented in the Appendix.

Dye Marking and Histology

Dye was iontophoretically injected into a cell following recording by either repeated pulses or a larger "blast"

of current, the latter being much more effective. Hyperpolarizing current pulses of 5-10 nA and 200 msec duration were applied to the electrode and cell at a frequency of 1 Hz, with periodic reversals of current direction to reduce electrode clogging. This method required over an hour to inject sufficient dye to show blue coloring of the cell, and the results were not consistent. A larger pulse of constant current (in the μA range) applied to the electrode for 1 to 5 minutes proved to be a more reliable method for injecting the dye. Occasionally, temporarily reversing the direction or briefly increasing the magnitude of the current were successful in "breaking" a clog in the electrode, thus restoring dye injection.

The tongue was fixed with either a 6% glutaraldehyde or an acid formalin solution shortly after dye injection. In some cases with surface cell recordings, the tissue was fixed with the electrode still in place to avoid detachment of the cell from the epithelium upon withdrawal of the electrode. Frequently, much of the dye would diffuse from the cell during glutaraldehyde fixation so that it was too faint to be located in section. This problem was eliminated by fixing the tongue with an acidic (pH about 5) formalin solution consisting of the following: stock formaldehyde (40%) solution, 10%; ethanol, 45%; acetic acid (galcial), 5%; deionized water, 40%.

The piece of tongue containing the dyed cell was fixed for 1 to 2 hours and then stored in a cacodylate buffer solution until it was histologically processed. After being dehydrated with acetone and embedded in epon, 5-7 μ thick sections were cut. The only counter-stains used that would not obscure the blue dye were combinations of acridine orange with eosin or methylene orange, applied to individual sections.

RESULTS

Electrical Properties of Lingual Cells

Electrical properties and response characteristics of taste bud cells and cells within the general epithelium of the tongue were studied and compared by means of intracellular recording. Certain differences in response characteristics to various chemical stimuli made it evident that at least three types of cells with distinct patterns of electrical responses could be distinguished among the cells examined. The surface epithelial cells not associated with taste buds all tended to give similar responses, and taste bud cells could be divided into two types each with certain unique response characteristics. However, responses to some stimuli were similar for cells in these different groups, and responses within a group varied somewhat from cell to cell. For this study, the cells are categorized as surface epithelial (SE) cells (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 (i.e., hyperpolarizations) from those of the other two cell types. The response differences are described in detail below.

In addition to their chemical responsiveness, these three types of cells differed in some of their electrical parameters. Penetration of any cell was signaled by an abrupt negative (relative to the reference electrode) shift in potential (the resting potential for that cell) while the microelectrode was being lowered by small steps into the tongue. As the electrode approached a deep epithelial cell or a taste bud obliquely through surrounding epithelium, several potential deflections were noted as the tip passed through the more superficial cells in the tissue; and the total deviation from zero potential was considered the deeper cell's resting potential. For taste bud cells, similar values were obtained by penetration directly at the surface or obliquely through surrounding tissue.

The resting potential, as measured shortly after penetration, for SE cells adapted to 0.1 M NaCl was -20.1±1.1 mV (mean ± S.E., n=67) with an input resistance of 26.5±2.7 megohms (n=39). Under these same conditions, resting potentials for the TB-1 and TB-2 cells were -39.4±1.6 mV (n=51) and -36.2±2.0 mV (n=21), respectively. Input resistance was 28.6±2.7 megohms (n=28) for TB-1 cells and 23.6±2.9 megohms (n=14) for TB-2 cells. The differences between the 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 were the input resistances of all three cell types significantly different from each other ($P>0.1$).

An approximation of the total cell membrane area was made for the general epithelial cells by assuming they are nearly spherical in shape with an average cell diameter of 10 μ (from personal observations). From this, a value of about 3.14×10^{-4} cm^2 was obtained; and this times the mean input resistance of these cells gives an areal specific membrane resistance of approximately 8.3 Kohm cm^2. Assuming that the taste bud cells have significantly larger surface dimensions, (see Figure 18), the areal specific membrane resistances of TB-1 and TB-2 cells would be larger than that of general epithelial cells since the input resistance values are similar.

The relationship between applied current and resultant membrane potential change was nearly linear for SE and TB-1 cells for the range of hyperpolarizing and depolarizing currents tested. In TB-2 cells, the I-V relationship was approximately linear for depolarizing and smaller hyperpolarizing currents; but the slope became nonlinear at hyperpolarizations of more than 20 mV from the resting level (Figure 2). The slopes of the I-V relationships varied from cell to cell, representing the different input resistances.

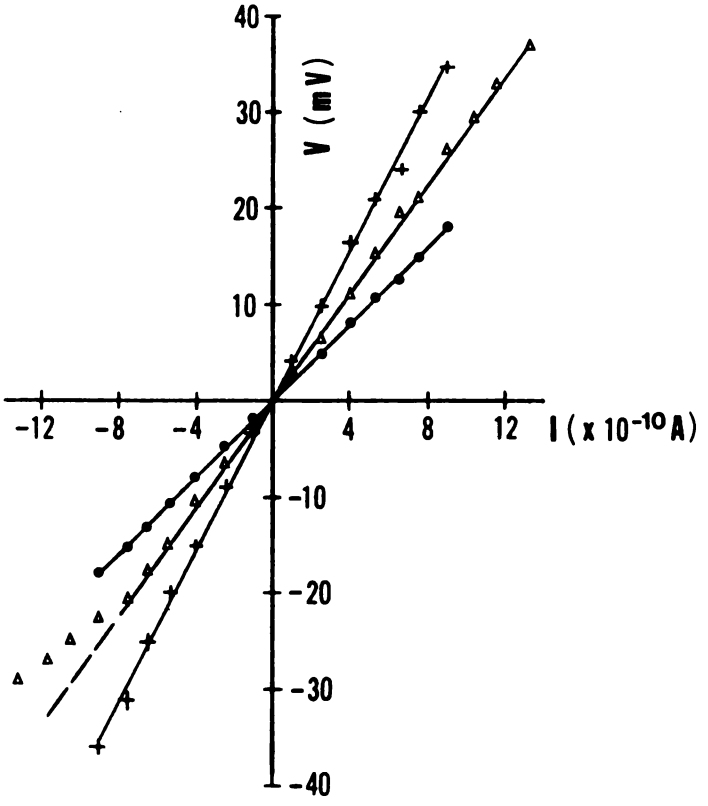

Figure 2. Current-voltage relationships obtained from representatives of the three types of cells. ● = SE cell; + = TB-1 cell; Δ = TB-2 cell. These cells had input resistances calculated from the linear portion of each slope of 20 megohms (SE), 38 megohms (TB-1), and 28 megohms (TB-2).

Responses of Lingual Cells to Various Salts and Water

SE Cell Responses

Applying a variety of salt solutions to the tongue often caused a change in the membrane potential of practically every surface epithelial cell that was examined. These potential changes were similar to responses to chemical stimulation reported for taste cells, and for this reason they are referred to in this study as receptor potentials, although these cells are not generally considered receptors. Eyzaguirre et al. (1972) found that "non-taste" surface epithelial cells in the toad respond to sapid stimuli, and this may be a general circumstance for non-keratinized lingual epithelium.

In general, monovalent cationic salt solutions more concentrated than the adapting 0.1 M NaCl evoked a depolarization; whereas, less concentrated salt solutions and water caused the SE cells to hyperpolarize. This was particularly evident for NaCl (Figure 3) and KCl solutions, which evoked relatively rapid and large membrane potential shifts. The magnitude of the response was proportional to the difference in concentration between adapting and test solution. The form of these salt responses was quite regular with the membrane potential rising or falling rapidly during the first several seconds of stimulation and then plateauing to a rather constant and maintained level. With either the

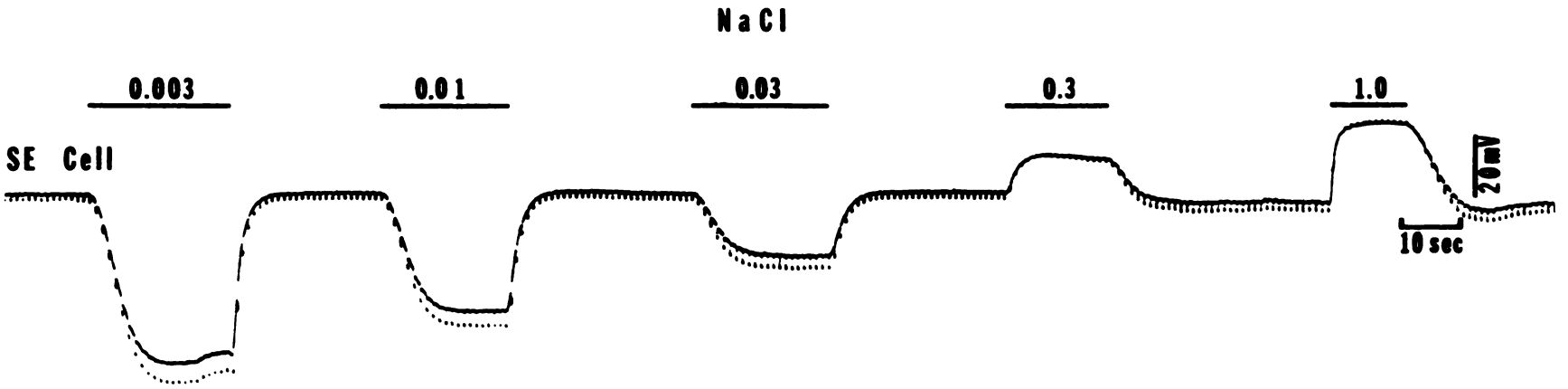

Figure 3. Responses recorded from an SE cell to NaCl solutions of increasing concentration. In this and following figures, the vertical deflections superimposed on the potential records represent the electrotonic potentials produced by a series of hyperpolarizing current pulses injected into the cell. The amplitude of this potential is proportional to cell membrane resistance (electrode resistance not included) at a ratio of 1 mV deflection = 10 megohms input resistance. Note that in this instance, resistance increases during hyperpolarization and decreases during depolarization. In all figures, horizontal bars above or below potential records indicate time of stimulus application.

rising or the falling phase of a response, depolarizations tended to be somewhat more rapid than the hyperpolarizations of that response (Figure 4).

Responses to water and lower concentrations of NaCl or KCl (e.g., 0.003 M) were quite large, often increasing the membrane potential by 50 mV or more; but these large membrane potentials usually were not well maintained, gradually decreasing in an irregular manner. Another characteristic of the SE cell response to water was an additional brief hyperpolarization of up to 25 mV at the offset of the water stimulus (Figure 5A). Following this rapid (1-10 sec) "off-response", the membrane potential would quickly approach its resting level.

Responses to NaCl and KCl were very similar (Figure 4); but the other monovalent cationic salts tested (NH_4Cl, Na_2SO_4, K_2SO_4) gave some variations in their evoked responses. For example, solutions of 0.1 M Na_2SO_4 (Figure 6) and K_2SO_4 would cause no potential change or a small one (7mV or less) of either polarity. Usually the responses of these two salts were alike, though not always.

NH_4Cl at higher concentrations was particularly effective in depolarizing the SE cells. In some instances, 0.03 M NH_4Cl would evoke a biphasic response consisting of a small, brief depolarization followed by a small (relative to other 0.03 M salts) hyperpolarization or a return to near baseline (Figure 4). Frequently, 0.1 M NH_4Cl caused a small

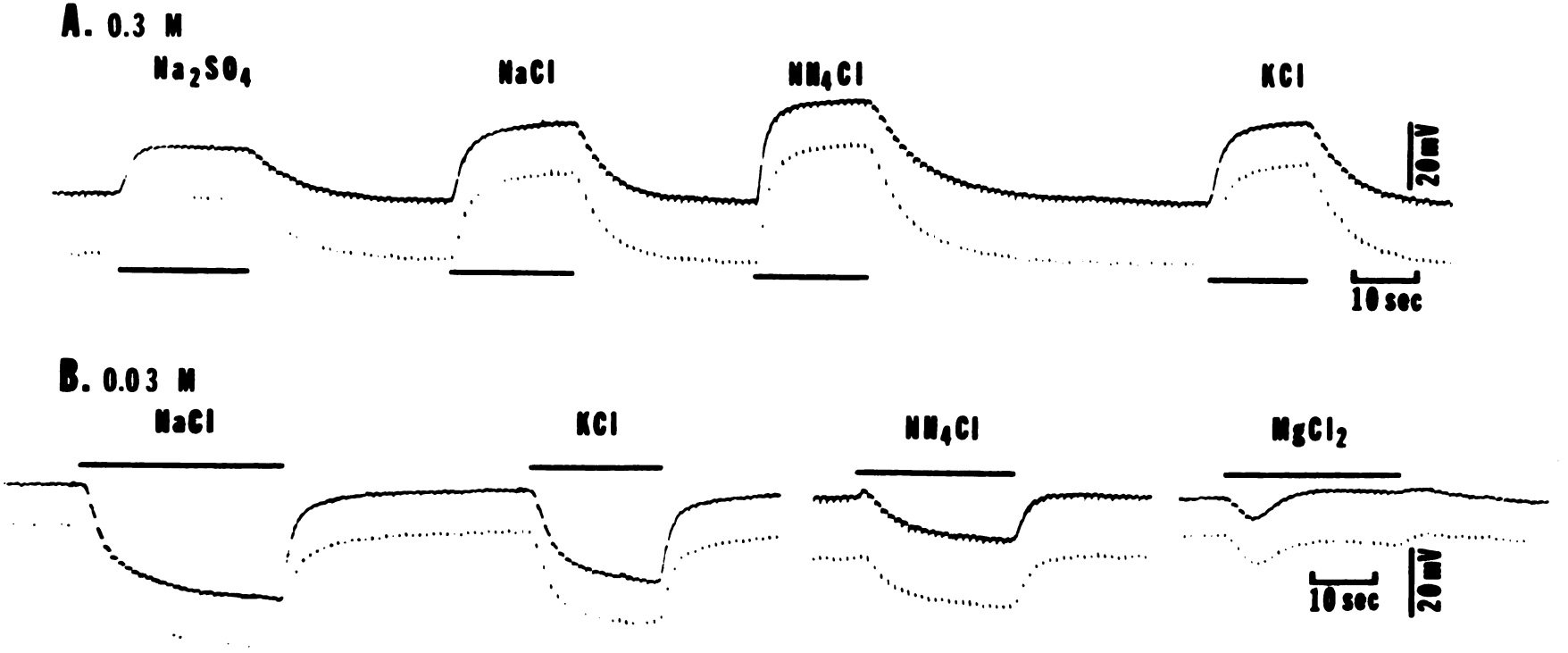

Figure 4. Responses from SE cells to various 0.3 M (A) or 0.03 M (B) salts. In this and all subsequent figures, each mV of electrotonic potential deflection = 10 megohms of system resistance (electrode + membrane input resistance).

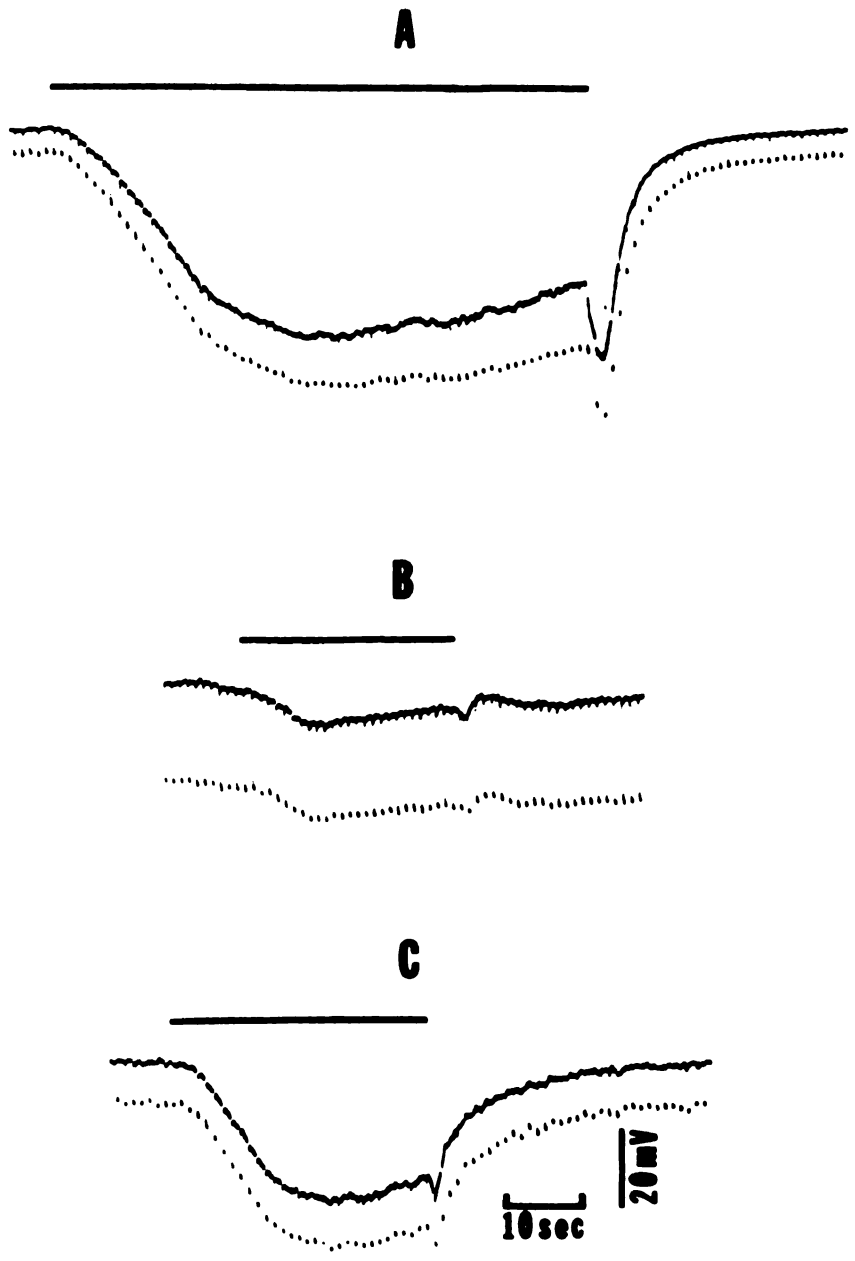

Figure 5. Hyperpolarizations by (A) SE, (B) TB-1, and (C) TB-2 cells in response to deionized water. Note brief additional hyperpolarizations following the stimulus in each cell.

depolarization (Figure 6); and at 0.3 M, this salt depolarized the cells more than the same concentration of the other salts (Figure 4).

$MgCl_2$ and $CaCl_2$ were the only divalent cationic salts tested. Due to extreme potential changes, highly variable results, and occasional irreversible effects on the cells, $CaCl_2$ could not be tested at the high concentrations (0.03, 0.1 M) of the other salts. The responses of SE cells to $MgCl_2$ were unique in amplitude and membrane resistance effects (see below). In every case, 0.1 M $MgCl_2$ caused depolarization of the cell; and sometimes even the lower 0.03 M concentration reduced the membrane potential. The effectiveness series for depolarization of SE cells by 0.1 or 0.3 M salts was $MgCl_2$ > NH_4Cl > NaCl = KCl > Na_2SO_4 = K_2SO_4.

There were membrane resistance changes associated with the responses to salts as measured by a continual resistance monitor. Most of the depolarizations by more concentrated salt solutions were accompanied by a decrease in membrane resistance; whereas, increased resistance was seen with the hyperpolarizations caused by the more dilute solutions. Membrane resistance change was proportional to the response magnitude for a particular salt, but there were slight differences among the monovalent salts (Figure 7). For example, KCl consistently caused a greater resistance

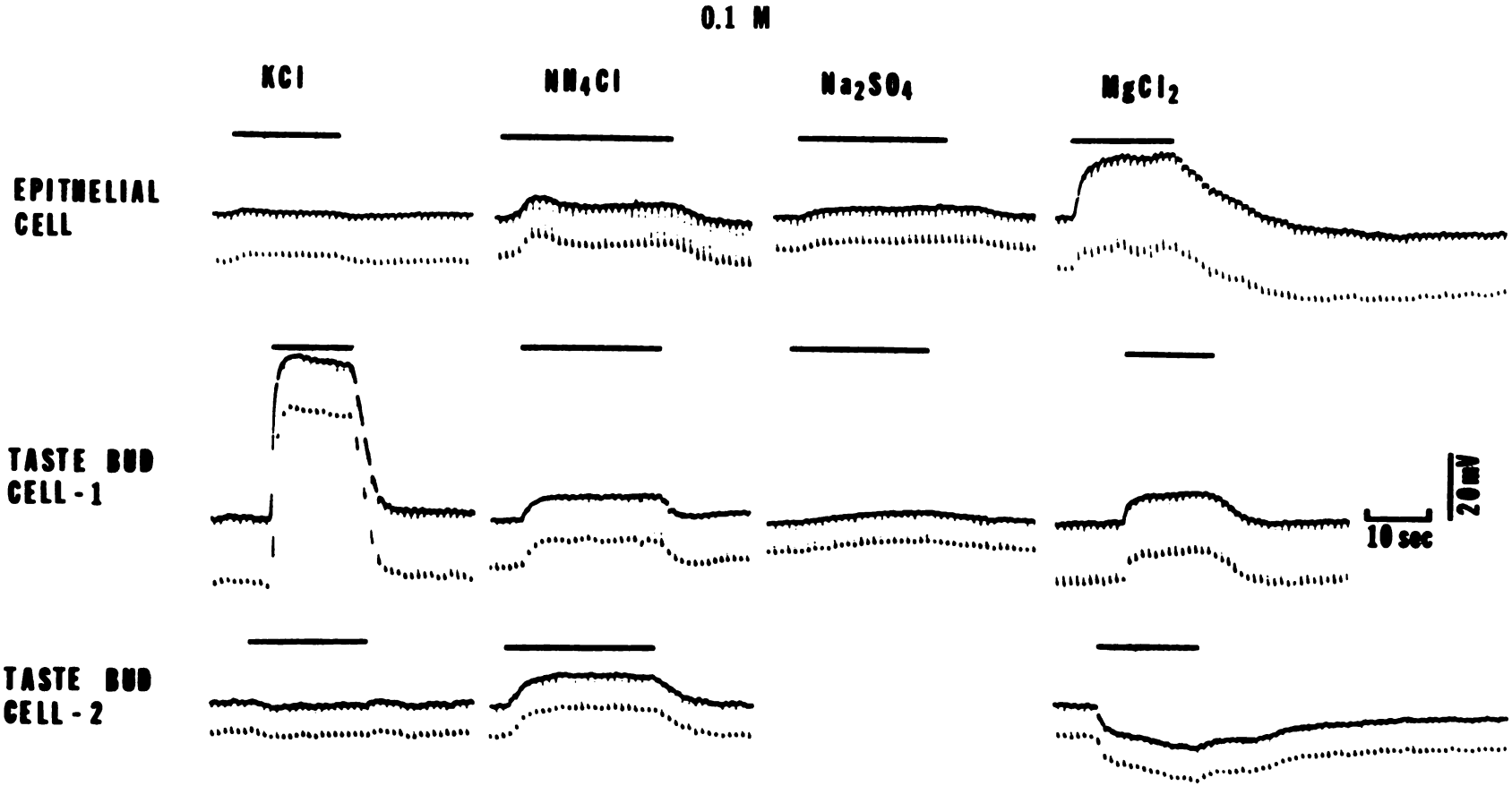

Figure 6. Comparison of responses to various 0.1 M salts in the three types of cells.

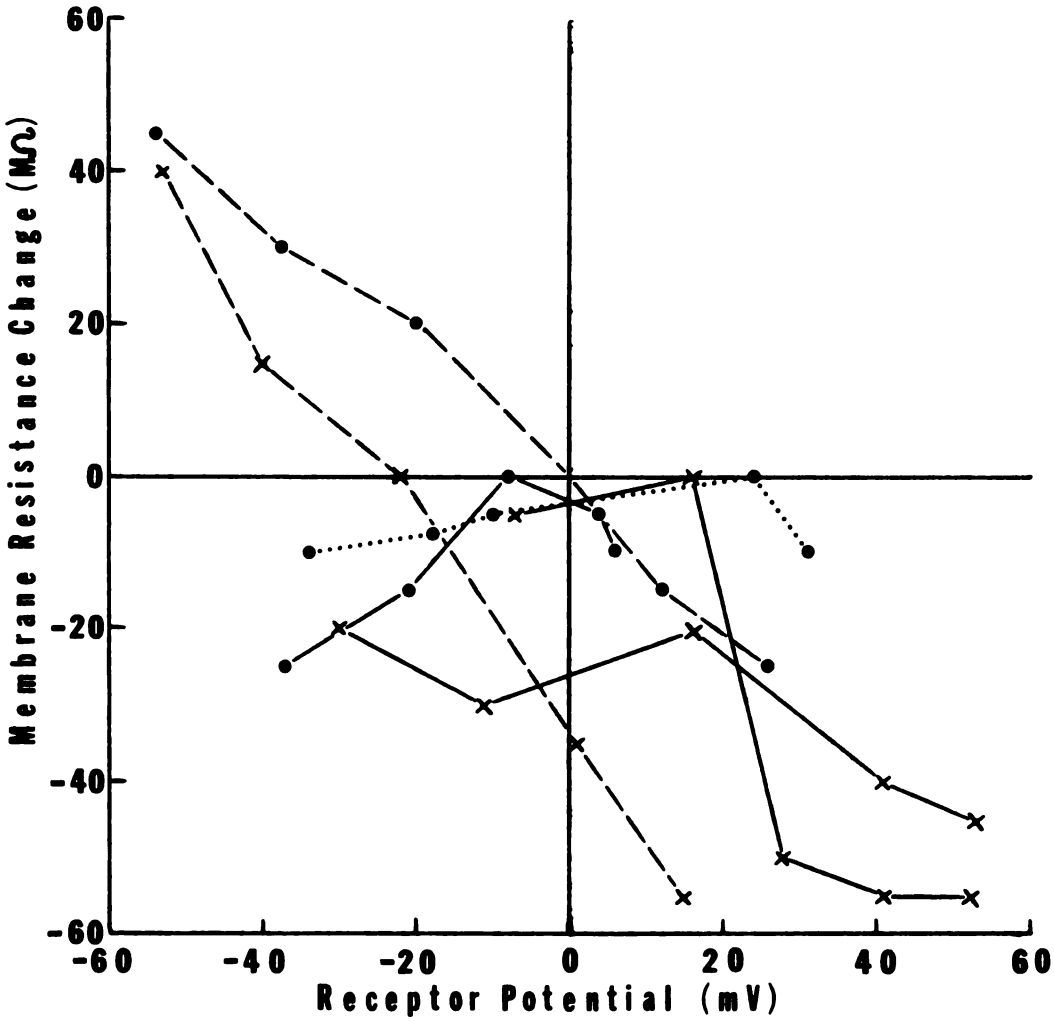

Figure 7. Relationships between receptor potential amplitude to salt solutions and membrane input resistance change for an SE cell (dashed lines), 2 TB-1 cells (solid lines), and a TB-2 cell (dotted line). ● = NaCl; x = KCl stimuli. Negative values represent hyperpolarizations and reductions in resistance.

change than NaCl at a particular response amplitude. A solution of 0.1 M KCl would occasionally decrease the membrane resistance with very small or no membrane potential change (Figure 6).

Unlike the other salts, $MgCl_2$ increased the membrane resistance even though at 0.1 M it caused large depolarizations (Figure 6). This resistance increase with $MgCl_2$ was several fold and, like the membrane potential, relatively slow (tens of secs) in returning to the resting level after stimulation. A smaller resistance increase was caused by 0.03 M $MgCl_2$ regardless of the polarity of the associated potential change (Figure 4).

TB-1 Cell Responses

The distinguishing characteristic of the responses of TB-1 cells was a depolarization to K-salts. Even at 0.03 M, K-salts evoked rapid depolarizations of these taste bud cells (Figures 8A, 9). At an equimolar concentration with the adapting NaCl solution (i.e., 0.1 M), K-salts often evoked extremely large depolarizations of 40 or 50 mV with rapid rise times (a few secs) and longer decay times after stimulation (Figure 6). These large responses were either maintained or partially phasic with a quick peak and a decrease of several mV to a maintained plateau.

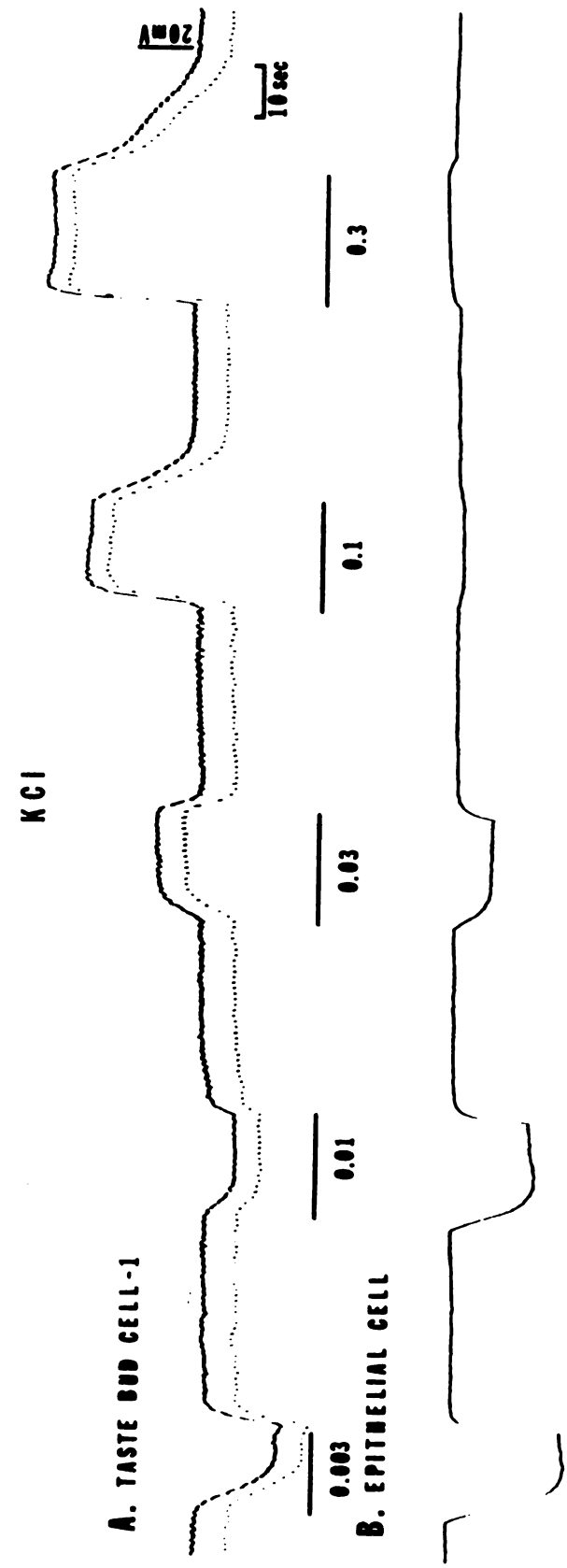

Figure 8. Responses recorded simultaneously from a taste bud (TB-1) cell (A) and an epithelial (SE) cell (B) to KCl solutions of increasing concentrations.

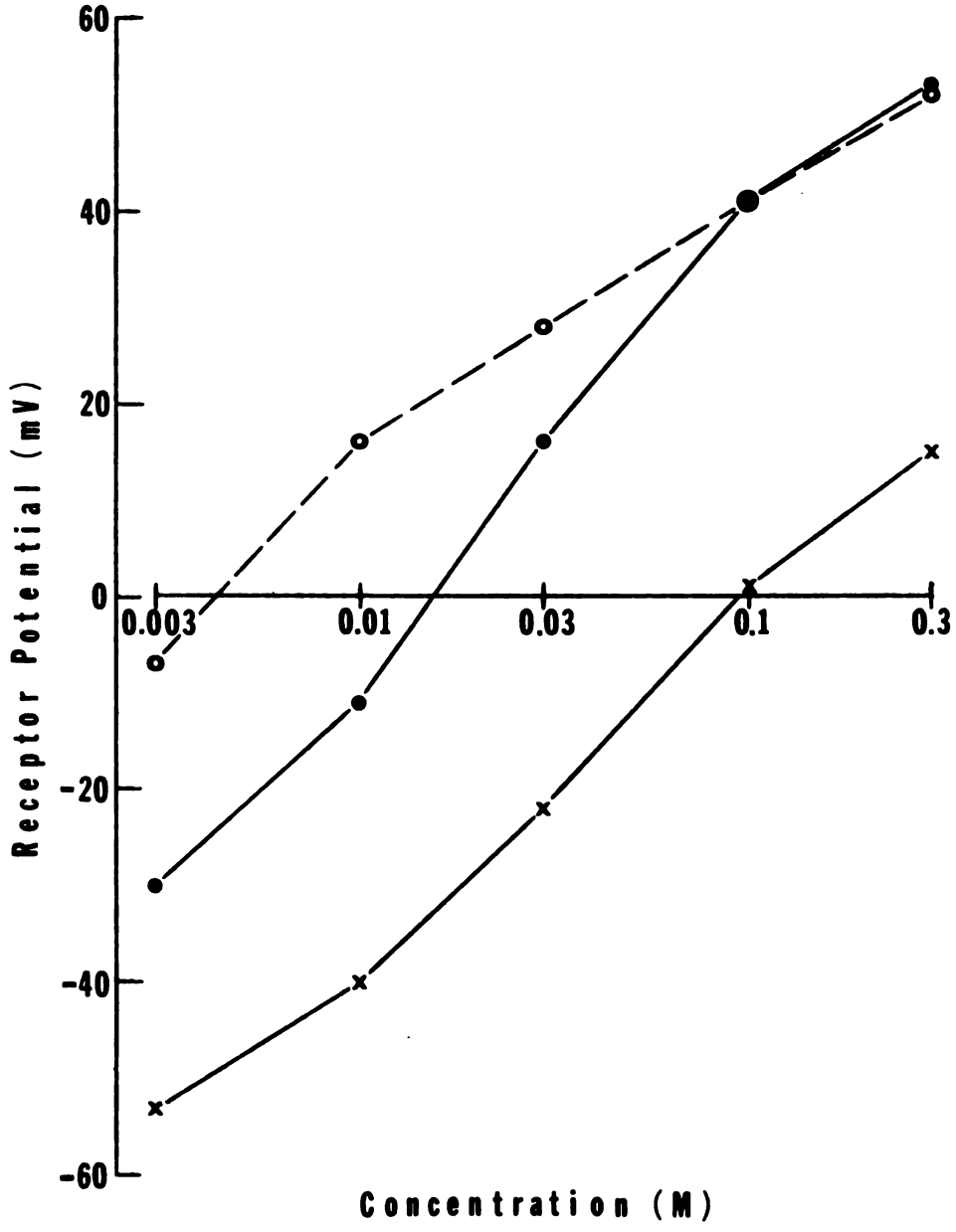

Figure 9. Relationships between concentration of KCl stimuli and receptor potentials in an SE cell (x's) and two TB-1 cells (filled and open circles). Note much greater depolarization to KCl in TB-1 cells than in SE cell.

Also, the TB-1 cells differed in their thresholds for depolarization to K-salts, cells giving responses of either polarity at 0.01 M. This difference appeared to depend on the resting potential of the cell at the time of stimulation since the same cell could hyperpolarize to 0.01 M KCl at one time and depolarize to the same stimulus at a (spontaneously) lower resting potential. However, they always depolarized to 0.03 M and hyperpolarized to 0.003 M K-salt solutions. All three K-salts tested (KCl, K_2SO_4, KI) were approximately equal in effectiveness though K_2SO_4 was occasionally more effective. At 0.1 M, these salts would depolarize the cell to the same potential level, regardless of membrane potential prior to stimulation (Figure 10).

The sensitivity of the TB-1 cells to NaCl was highly variable; some cells responded much like SE cells while others gave almost no response even to 1.0 M NaCl or H_2O. If a response to NaCl or other salt solution was evoked, it was like that of SE cells in which concentrated solutions depolarized and dilute solutions hyperpolarized the cell. TB-1 cells usually depolarized to 0.1 M NH_4Cl or $MgCl_2$ (Figure 6).

There were changes in the membrane resistance of TB-1 cells associated with some receptor potentials. In particular, there was a decrease in membrane resistance with the large depolarizations to 0.1 or 0.3 M K-salts. However, most stimuli, including $MgCl_2$, caused no or extremely small

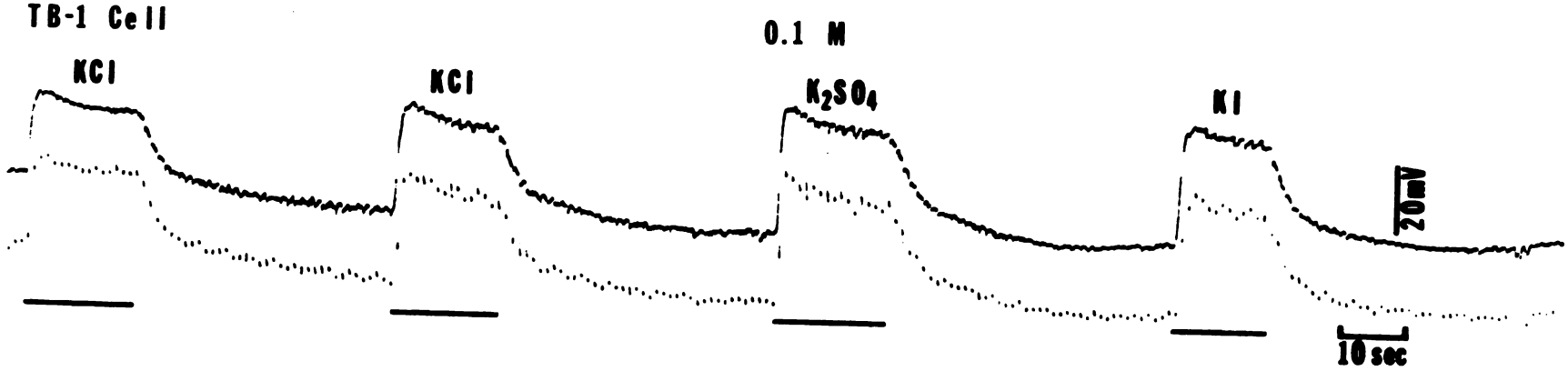

Figure 10. Responses recorded from a TB-1 cell to three 0.1 M K-salts. Note that the cell depolarizes to nearly the same potential in each case, even when initial resting potential was different (KCl).

changes in the membrane resistance, in contrast to the SE cells.

TB-2 Cell Responses

The membrane potential changes in TB-2 cells to NaCl and KCl solutions were indistinguishable from those in SE cells, though at times the membrane resistance changes were much smaller or of the opposite polarity (e.g., increased resistance with depolarization). Responses to other salt solutions were also similar to those of SE cells, yet the associated conductance changes were always very small or absent. If the TB-2 cell responded to NaCl solutions, it would hyperpolarize to water, with a small increase in membrane resistance, like the SE cells and would give a small additional "off-response" hyperpolarization, seen in all three types of cells, after their response to water (Figure 5).

A slow hyperpolarizing response to 0.1 M $MgCl_2$ solutions was characteristic of TB-2 cells, making them easily distinguishable from the other two types of cells. These responses had a generally slow time course, especially after stimulation when the membrane potential slowly and irregularly returned to near the resting value. In some cases, the membrane remained hyperpolarized, which altered the subsequent responses to this or other stimuli. During recording from TB-2 cells, the membrane potential gradually

decayed to near zero in several cases, with no apparent sudden potential shift signaling the electrode leaving the cell. During this condition, 0.1 M $MgCl_2$ would evoke a slow depolarization somewhat similar to that in SE cells; however, it was not possible to determine whether the electrode had left the cell or had in some way altered its responsiveness.

Comparison of Responses to Salts

When the microelectrode was directed to the taste bud, application of a 0.03 M or 0.1 M K-salt would clearly reveal TB-1 cells by the resultant depolarization response (Figures 9, 11). The TB-2 and SE cells would hyperpolarize to 0.03 M KCl and yield nearly no potential change to 0.1 M KCl.

The best single stimulus for categorizing cell type was 0.1 M $MgCl_2$ (Figure 6). In SE cells, a significant increase in membrane resistance always accompanied the depolarizing receptor potential. If only the membrane potential were monitored, the depolarization to $MgCl_2$ would not be different enough to distinguish TB-1 from SE cells; the lack of conductance change, though, in the TB-1 cell response made differentiation possible. The hyperpolarization to 0.1 M $MgCl_2$ in TB-2 cells made their responses unique.

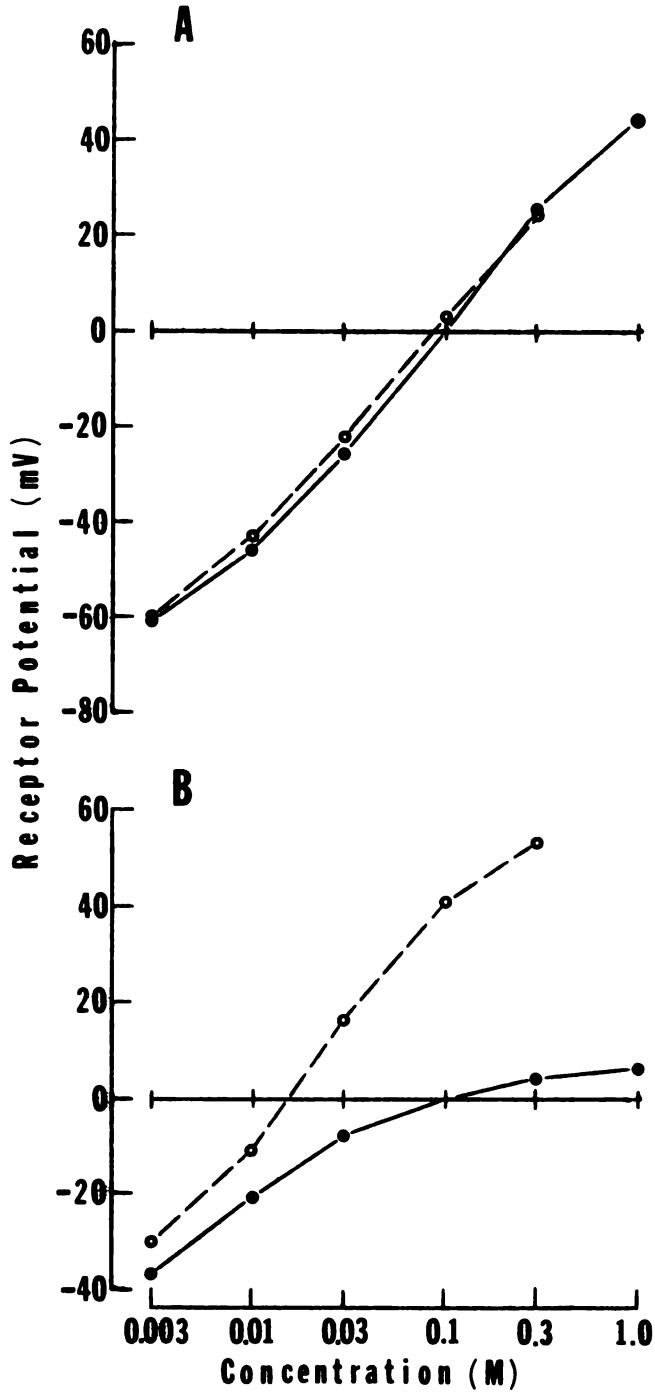

Figure 11. Relationships between NaCl (●) or KCL (o) stimulus concentration and receptor potential amplitudes for an SE cell (A) and a TB-1 cell (B).

Responses of Lingual Cells to the Other Basic Taste Qualities

Acid Stimulation

Acid solutions (HCl and H_2SO_4 equally) produced responses in SE cells that were perhaps the most dramatic for that cell type. Even the lowest concentration tested, 0.3 mM, evoked large depolarizations with a concurrent large increase in the membrane resistance (Figure 12B). Although the rate of rise of the acid response was often rapid, the onset of this response was usually delayed by several seconds with respect to stimulus onset, and occasionally it was preceded by a small hyperpolarization. Both the membrane potential and resistance were extremely slow to return to the resting level, taking tens of seconds to several minutes to recover from even a brief (e.g., 5 sec) stimulus. It was interesting to note that during this falling phase of the acid response, other stimuli could temporarily reduce the enormous resistance caused by the acid (e.g., 0.1 M NH_4Cl).

More concentrated acid solutions caused faster rise times, in some cases less than 200 msec to near peak depolarization. The magnitude of the acid response was increased only slightly by increases in concentration, but the resistance change was increased significantly and the delay time was decreased at higher concentrations.

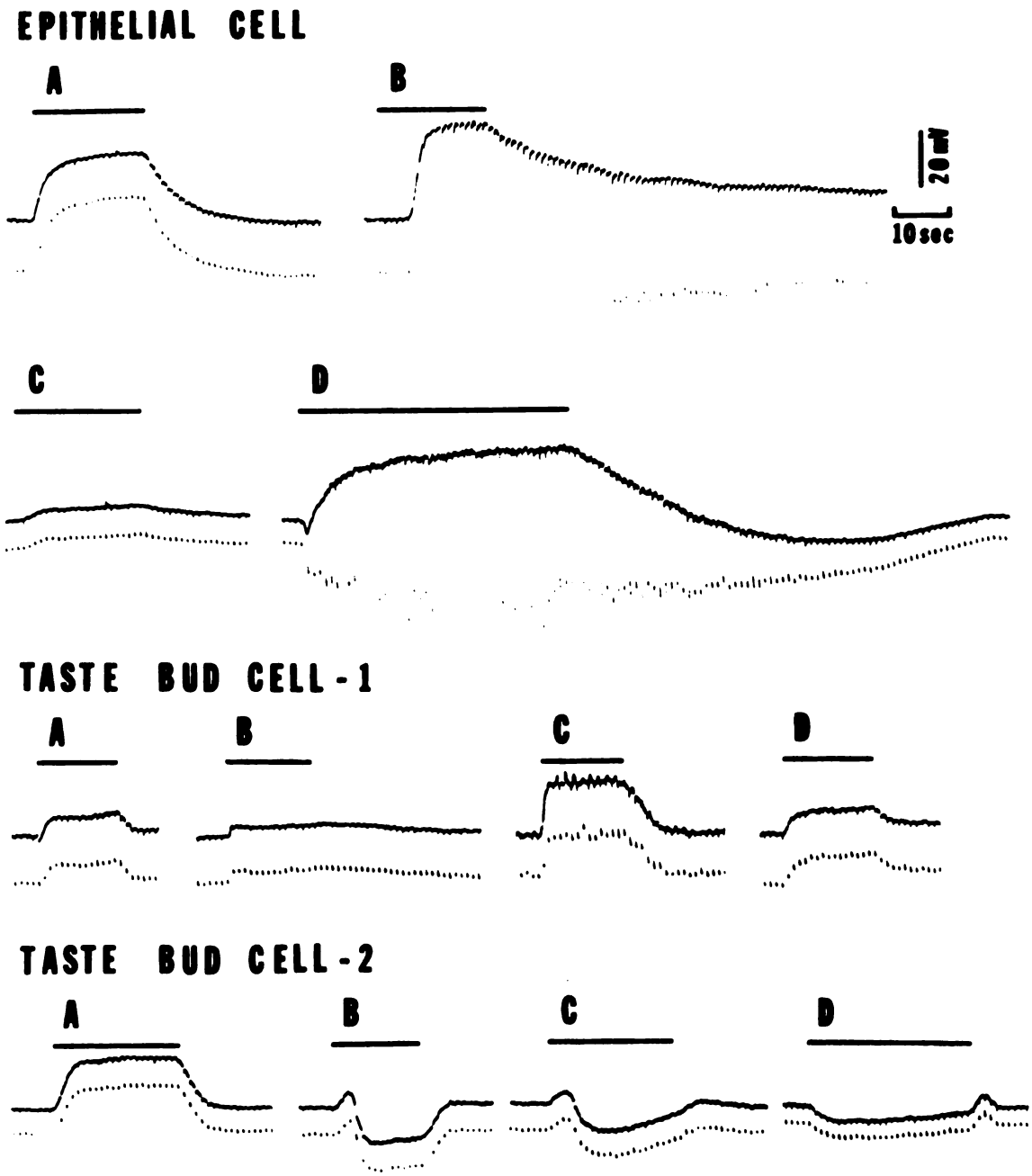

Figure 12. Responses from three types of cells to the different taste qualities. Stimuli were 0.3 M NaCl (A), 0.0003 M H_2SO_4 (B), 0.3 M sucrose (C), and 0.003 M QHCl (D).

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 within a particular cell, appeared to be due to the change in the response at different membrane potential levels. For example, in one TB-1 cell, initially there was no acid response; but after the membrane potential spontaneously increased by 40 mV, an acid response of 40 mV was obtained. When present, these depolarizations were quick to rise but slow to return to baseline.

In TB-2 cells, acid solutions primarily evoked a hyperpolarizing response, unmistakably different from the depolarizing responses of SE and TB-1 cells (Figure 12B). Most of these responses were biphasic, consisting of an initial brief depolarization immediately followed by a hyperpolarization that gradually but consistently decayed toward the resting membrane potential. Following the stimulus, the TB-2 cell membrane potential would depolarize to or beyond the initial resting level; and in most cases, it would return to the resting potential within one minute. Increasing the concentration of the acid solution increased the magnitude of the hyperpolarization.

Each type of cell responded differently to acid solutions (H_2SO_4 or HCl), making this another valuable test for discrimination. Both SE and TB-1 cells gave depolarizations

that were slow to decay, but only the SE cell dramatically increased its resistance. The TB-2 cells tested with acid (12 cells) consistently gave a response of the opposite polarity, setting them apart from the other cells (Figure 12B).

Sugar Stimulation

Concentrated sucrose solutions (0.1 M and 0.3 M) were ineffective in evoking membrane potential or resistance changes in many SE cells. Other cells (n=10) did respond with a small (few mV) depolarization (Figure 12C) or rarely with a hyperpolarization. When they occurred, these responses were moderately slow to develop and showed no measurable conductance change. The most likely time to find a sucrose response was shortly after penetration of an SE cell, suggesting that this response may be labile due to preparation decay or effects of other stimuli. A few tests with fructose gave results similar to those for sucrose.

The responsiveness of TB-1 cells to sucrose depended on the membrane potential, as it did for acid. When present, the response to sucrose usually was a small depolarization with no or 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 (Figure 12C). Membrane resistance changes were negligible with sweet stimuli.

Quinine Stimulation

Quinine (QHCl or QSO_4) produced variable membrane potential changes in SE cells. Of the two concentrations tested, the lower (0.003 M) gave the most unpredictable potential changes, causing either slow depolarizations or brief hyperpolarizations, both with a slow return to resting potential. The more concentrated (0.03 M) solution usually evoked a slow depolarization of the cell with a very prolonged repolarization requiring tens of seconds. If any potential change was evoked by quinine, it was always slow, taking many seconds to occur.

A characteristic effect of quinine on the SE cell was an enormous increase in membrane resistance to many times the resting level (Figure 12D). This resistance change was progressive and began at the onset of the stimulus regardless of the potential change. After cessation of stimulation, the resistance gradually decreased, and there frequently was an associated slow post-stimulus hyperpolarization of the cell before the return to the resting potential. The resistance increase was greater and faster with the more concentrated quinine solution; if 0.003 M QHCl evoked a depolarization, then 0.03 M QHCl always caused a larger one.

Rather small depolarizations were evoked by QHCl solutions in some TB-1 cells (Figure 12D), 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

It is evident from the previous description that the characteristics of the responses to chemical stimulation differed sufficiently among cells to warrant their categorization into three groups. The responses of the different cells within one group also varied, but there were certain similarities that made it possible to reliably place a cell within one of the categories. Some responses were similar for the different cell types (e.g., to NaCl or KCl solutions for SE and TB-2 cells, Figures 12A, 13) so that these cells could not be differentiated by these responses alone. Other stimuli consistently yielded responses sufficiently distinct to allow discrimination of the cells. The following description briefly emphasizes the responses that characterize each type of cell. The membrane potential changes are compared by the response profiles of the cells within each group (Figure 14), and Table 2 summarizes the major differences for the three types of lingual cells.

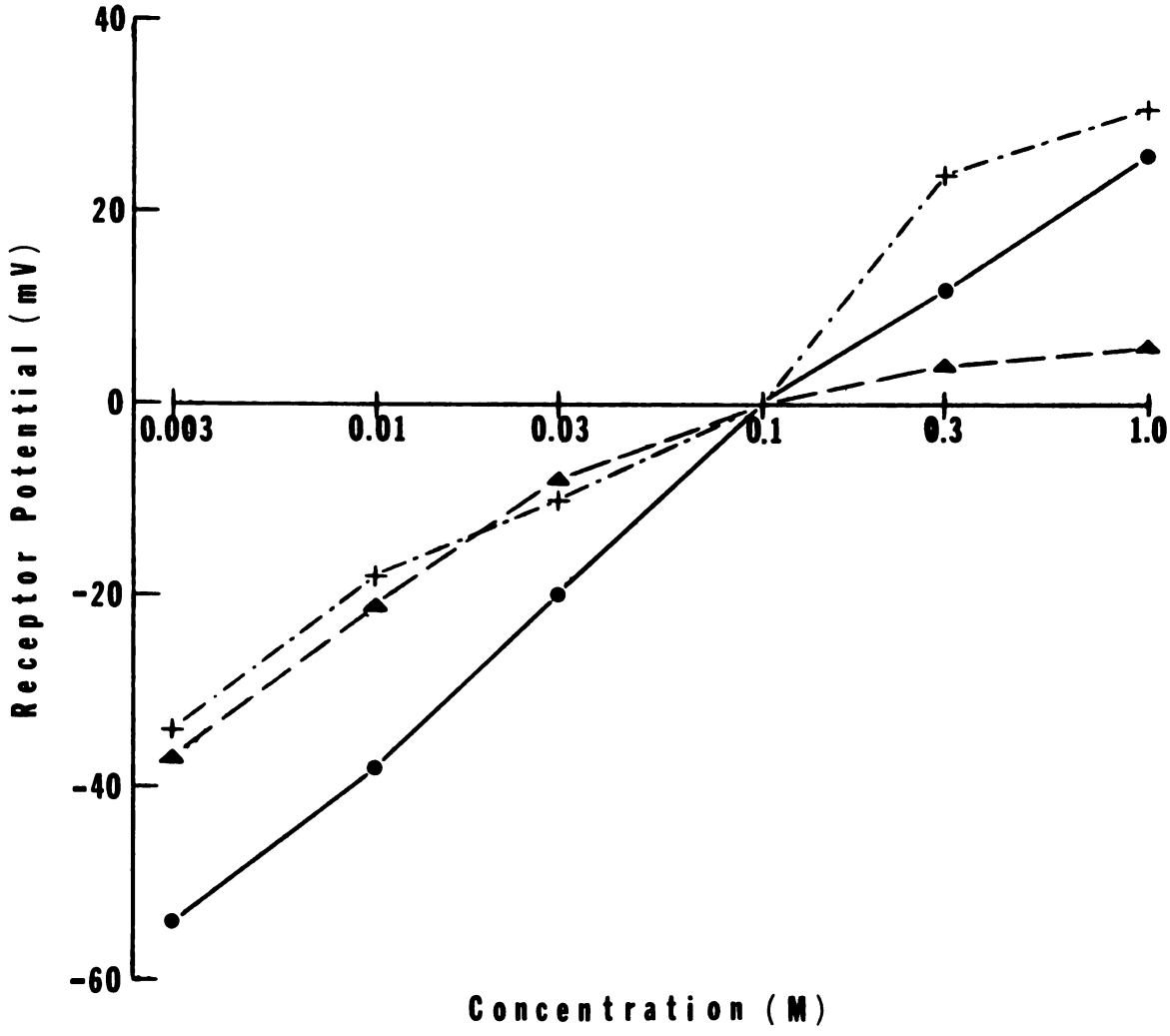

Figure 13. Relationships between NaCl stimulus concentration and receptor potential amplitudes of an SE cell (●), a TB-1 cell (▲), and a TB-2 cell (+). Same TB-1 cell values were used in Figure 11B; NaCl receptor potentials from a different SE cell were used in 11A.

Figure 14. Response profiles of 12 SE cells, 8 TB-1 cells, and 6 TB-2 cells to various stimuli. Depolarizations and hyperpolarizations are presented by positive and negative values, respectively; x 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 $MgCl_2$ for TB-2 cells due to the significance of these stimuli (see text).

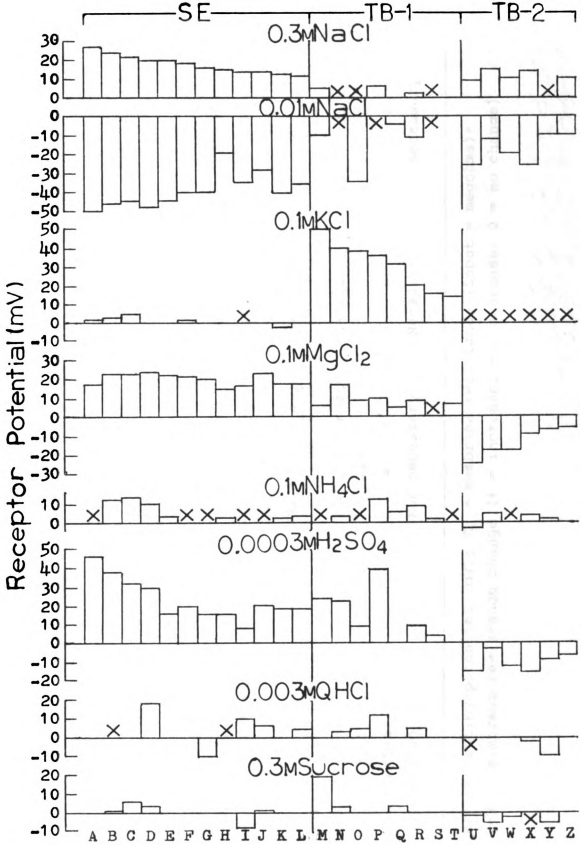
SE
TB-1
TB-2
0.3MNaCl
0.01MNaCl
0.1MKCl
0.1MMgCl2
0.1MNH4Cl
0.0003MH2SO4
0.003MQHCl
0.3MSucrose
Receptor Potential (mV)
A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

Figure 14

Table 2. Summary of Differences for the Three Types of Cells

	SE	TB-1	TB-2
Mean R.P.	-20.1± 1.1	-39.4± 1.6	-36.2± 2.0
R.P. range	-6 to -40	-20 to -64	-22 to -60
Mean input R_M	26.5± 2.7	28.6± 2.7	23.6± 2.9
Input R_M range	10 to 70	15 to 60	15 to 50
Specific R_M	8300 ohm cm^2	>8300 ohm cm^2	>8300 ohm cm^2
Effectiveness series	$MgCl_2$>>NH_4Cl> NaCl = KCl> Na_2SO_4 = K_2SO_4	K_2SO_4 = KCl>> $MgCl_2$ = NH_4Cl> NaCl > Na_2SO_4	Variable
Electrical Coupling	+	-	+
C-R_M (Salts)	$MgCl_2$ ++; rest-	K-Salts-; rest 0	All salts 0
C-R_M (QHCl)	+++	0	0
C-R_M (Acids)	++	0	0
Circulation	Not necessary	Necessary	Necessary

R.P. = resting potential (mV); R_M = membrane resistance (input = megohms);
C-R_M = membrane resistance change (+ = increase, - = decrease, 0 = no change)

SE Cells

The SE cells adapted to 0.1 M NaCl were particularly sensitive to variation of salt concentration of the bathing fluid, as revealed by membrane potential and resistance changes. Generally for salts other than $MgCl_2$, concentrated solutions decreased the membrane potential and resistance of SE cells while dilute solutions had opposite effects.

Many stimuli produced large changes in SE cell membrane resistance including quinine which increased the resistance with or without a potential change. Similar increases in membrane resistance were evoked by acid and 0.1 M $MgCl_2$ solutions, along with large (10-50 mV) depolarizations.

TB-1 Cells

TB-1 cells were readily identifiable by their rapid depolarizing responses to K-salt solutions above 0.01 M. These responses were relatively large (e.g., 54 mV to 0.3 M KCl in Figure 8) and consistent, unlike the responses of TB-1 cells to other salts, which were variable in size and occurrence.

It should be emphasized that the receptor potentials evoked by acid, quinine and sugar stimuli in TB-1 cells appeared to be strongly dependent upon the membrane potential level at the time of stimulation. This phenomenon was expecially evident for the responses to acid solutions. In many cases, responses were absent or quite small when

the resting potential was low but appeared as depolarizations when a higher resting potential value was recorded. However, the responsiveness of TB-1 cells to the four taste qualities was independent, since large receptor potentials were recorded for some qualities and not for others even at the same resting membrane potential. Therefore, the degree of sensitivity of TB-1 cells for the different qualities varied from cell to cell in addition to the dependence upon the amplitude of the resting potential for the overall sensitivity of each cell.

The TB-1 cell membrane resistance changes were small for practically all responses. Membrane resistance tended to increase with sucrose and acids and to decrease with concentrated K-salt and quinine stimulation.

TB-2 Cells

Most salt stimuli evoked potential changes in TB-2 cells indistinguishable from those for SE cells (i.e., they depolarized to concentrated, hyperpolarized to dilute solutions). Alternatively, 0.1 M $MgCl_2$, acid, and sweet solutions produced hyperpolarizations in these cells, in constrast to the depolarizing responses of SE and TB-1 cells. A common characteristic of all TB-2 cell responses was the relatively small concurrent change in membrane conductance, if any occurred, quite different from the conductance changes observed for SE cells.

It is possible that other cell types, perhaps less accessible for intracellular recording, exist within the tongue epithelium. In addition, these three categories might be further subdivided by more extensive testing. Unresponsive cells or cells responding much like SE cells may be present in the taste buds along with the TB-1 and TB-2 cells, but this has not been determined.

Effects of the Adapting Solution

In most of the experiments, the tongue was adapted to a 0.1 M NaCl solution continuously flowing over the recording region. Since NaCl is a sapid substance, it had some influence on the cells' electrical parameters and responses. Therefore, several experiments were conducted using less concentrated adapting fluids.

For both the SE and the TB-1 cells tested, adaptation to 0.01 M NaCl yielded slightly higher resting potentials, though not enough cells were tested to show statistically significant differences. Also, the responses to NaCl solutions were altered in a predictable manner, with the division between hyper- and depolarization responses still being the concentration of the adapting solution.

SE cells gave large depolarizations to 0.1 M NaCl, and other depolarizing responses to concentrated salts were proportionately enhanced. Likewise, hyperpolarizations to

NaCl or KCl solutions less concentrated than 0.01 M were smaller and had a slower time course. In general, the same order of effectiveness to salts was maintained. When adapted to amphibian Ringer saline, the SE cells yielded basically similar results as under 0.1 M NaCl adaptation. A Ringer test during 0.1 M NaCl adaptation gave a small depolarization of a few mV.

In TB-1 cells, adaptation to a more dilute solution had an effect similar to that in the SE cells. Switching to a water rinse after the 0.1 M NaCl increased the resting potential of a TB-1 cell by about 19 mV. When adapted to water, this cell yielded a 16 mV depolarization to 0.1 M NaCl; but the depolarization to 0.1 M KCl was nearly twice as large (30mV). The importance of these findings is that it proves that TB-1 cells actually were more sensitive to KCl than to NaCl solutions, and that the larger responses to KCl 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 pairs of SE cells, though not all adjacent cells were found to be coupled (Figure 15A). Coupling tended to be weak, with coupling ratios averaging about 0.1 and not

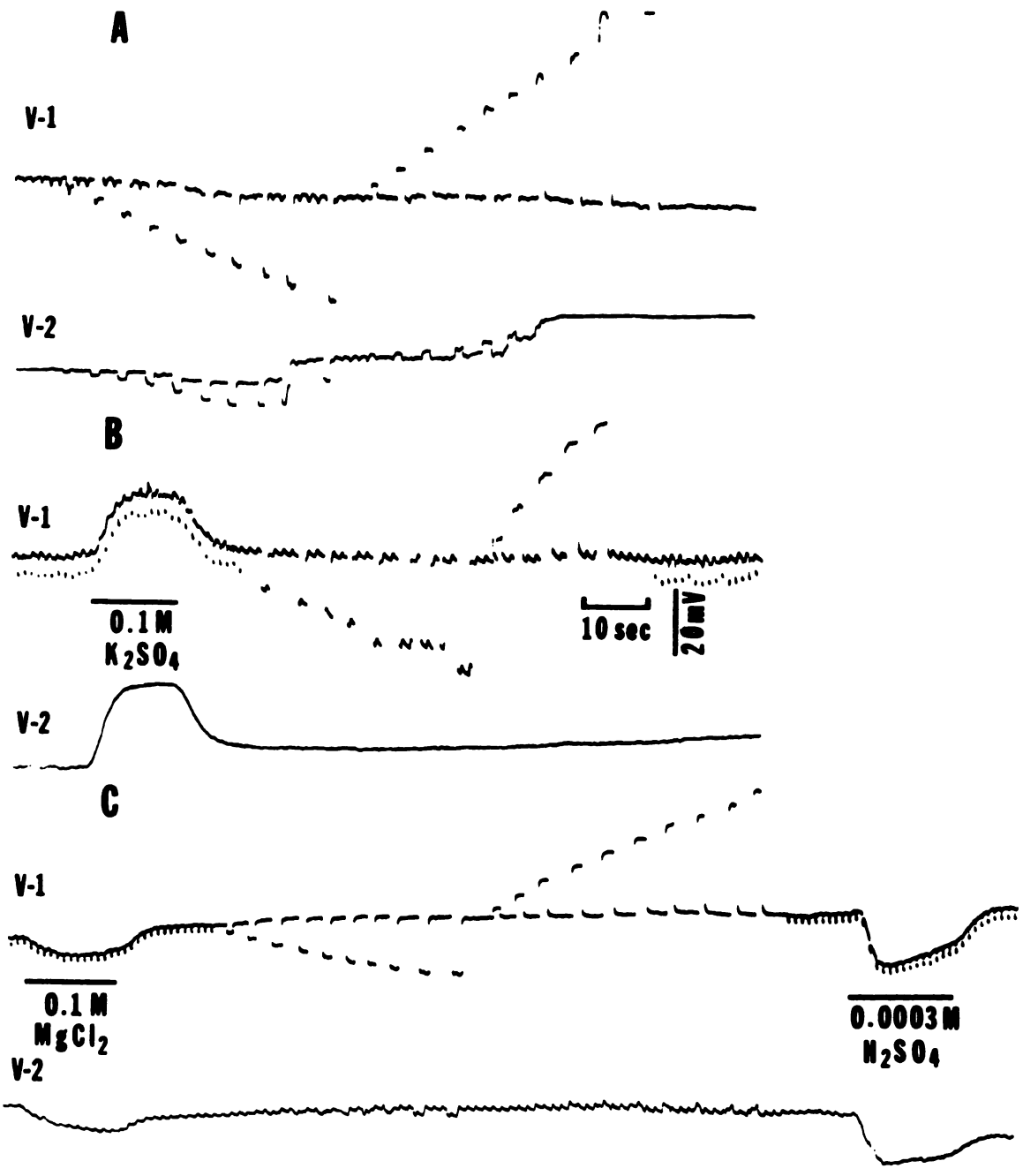

Figure 15. Test for electrotonic coupling between pairs of SE cells (A), TB-1 cells (B), or TB-2 cells (C) by comparing voltage changes in current-injected cell (V-1) with those in neighboring cell (V-2). Coupling ratios (V-2/V-1) were A = 0.28, B = 0, C = 0.11. The progressive loss of V-2 potential in A indicates electrode leaving that SE cell with concurrent loss of the electrotonic potentials that indicate coupling.

exceeding 0.3 (e.g., 0.28 for cells in Figure 15A). Although these coupling ratios are rather small, they were consistently observed for many pairs of SE cells (Figure 16), and the values are high enough to make the possibility of their being recording artifacts quite unlikely.

The necessity of simultaneous recordings from small cells adjacent to each other made coupling tests difficult to perform in this tissue. Even small mechanical disturbances could dislodge one or both electrodes. This factor, along with the inherent cell damage caused by the electrodes, may be the reason that coupling between two cells usually decreased within minutes after penetration in most cases. Also, coupling was more easily demonstrated at the beginning of an experiment, suggesting that these intercellular connections were susceptible to disruption with time.

Additional evidence for intercellular communication was provided by the injection of Procion navy blue dye into these cells. When the dye was successfully injected, it could be visualized through the dissecting microscope in situ. In many cases, the injected dye spread into one or more of the neighboring epithelial cells, possibly through the same connecting channels that allowed movement of the current-carrying ions (see Literature Review). Dye spread and electronic coupling were found for both deep and surface epithelial cells (see Figure 17B).

Figure 16. A. Location on tongue of sites of electrotonically coupled pairs of SE cells (o) or TB-2 cells (●). Dashed line indicates approximate position of overlaying upper jaw which limited the recording area. This is a summary from 22 experiments.

B. Oscilloscope trace of recordings from coupled SE cell pair with a coupling ratio of 0.15. Calibration marks: vertical = 10 mV; horizontal = 5 msec.

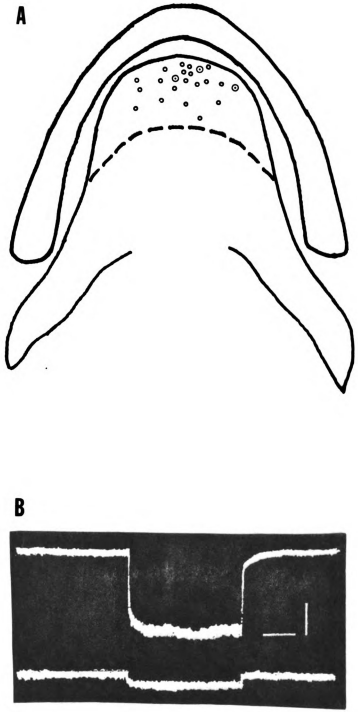

Figure 16

Coupling was also tested between taste bud cells, and TB-2 cells had coupling ratios similar to those for SE cells (e.g., 0.11 for cells in Figure 15C). In each instance of simultaneous recordings from two TB-2 cells within a bud, some coupling was observed even though the degree was very small in one instance (probably because coupling was through a third, intermediate cell). Coupling was monitored by repeated current pulses for several minutes in one pair of TB-2 cells during the application of several chemical stimuli, and it did not change significantly during stimulation.

Electrically coupled pairs, whether SE or TB-2 cells, always gave very similar responses to chemical stimuli. At times, the potential levels clearly paralleled each other, but in some cases the receptor potentials were smaller in one member of the pair. Roughly speaking, the resting potentials were nearly the same in coupled partners. Current passed well in either direction between coupled cells of both types indicating non-rectifying electrotonic synapses.

Several recordings from pairs of TB-1 cells were obtained, but no coupling was observed in any instance (Figure 15B). This finding suggests, but does not prove, an absence of coupling between TB-1 cells. Negative findings were also obtained between cells of different types; however, it was never shown that these cells were truly neighboring so that this finding is uncertain.

Control experiments in which large current pulses were passed with one electrode outside the cell while recording intracellularly with the other yielded negative results. An example can be seen in Figure 15A, where the electrotonic potential changes in the follower cell (V-2) decreased and disappeared with the loss of intracellular placement of the electrode as evidenced by the loss of recorded membrane potential. In addition, successful 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 following intracellular recording served two purposes. First, it was a reliable indicator that the tip of the electrode was actually inside a cell. This is particularly important for recordings during chemical stimulation because of the possibility of physicochemical artifacts (see Appendix). Secondly, dye marking allowed histological identification of the type of cell recorded from and its location.

Most of the electrodes used for this study were filled with a Procion navy blue dye solution that was electrophoretically injected into the cells following recordings. 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.

Due to the loose adhesion of the surface cells in the mudpuppy tongue, the SE cells would frequently detach from the epithelium when the electrode was withdrawn unless the tongue was previously fixed with electrode still in place. Therefore, clear histological localization of SE cells was rare. One example is shown in Figure 17A, where the dye was localized in the cell, apparently close to the nucleus.

Deep epithelial cells, that gave small or no responses to chemical stimulation, were more readily retained within the tissue, thus allowing subsequent identification in section. Figure 17B shows one obviously stained (blue) cell with some dye in an adjacent cell. The localization of the dye in two cells may represent dye spread between electrotonically coupled cells.

In several instances, the dye was injected into taste bud cells (5 TB-1 and 3 TB-2 cells) and subsequently identified in section. One such example is shown in Figure 18A, which shows the blue dye again around the cell nucleus. During recording, this cell responded as a TB-1 cell. In this section, the pink counter stain reveals the underlying

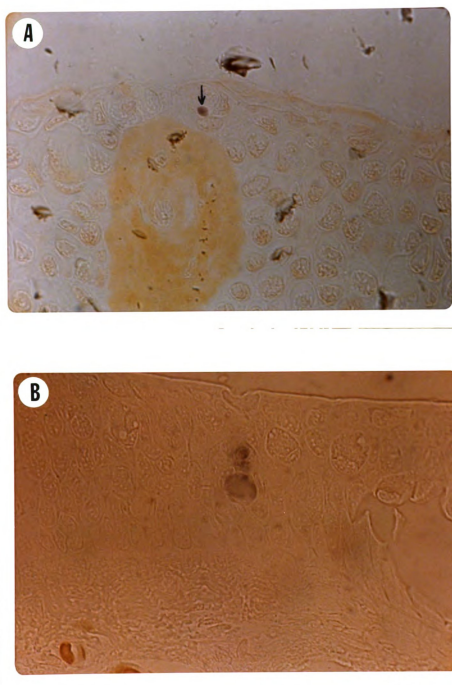

Figure 17. Histological identification of recording site in surface (A) and deep (B) epithelial cells by injected Procion navy blue dye. Dye was localized near the nucleus of an SE cell (A, indicated by arrow) but spread to 2 cells in B. Counterstain in A was methylene orange.

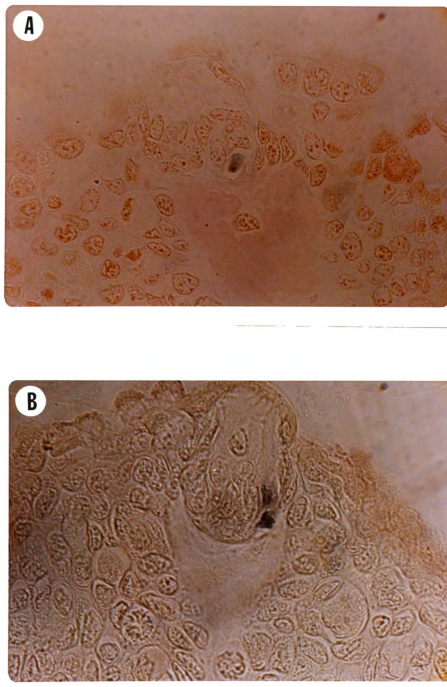

Figure 18. Histological identification of dye-injected TB-1 (A) and TB-2 (B) cells. Note blue dye in A is near cell nucleus in base of bud and in B is in 2 cells near periphery of bud. Counterstain in A was eosine (pink dermal papilla) and acridine orange (cell nuclei).

dermal papilla that is found beneath each taste bud. This landmark and the oval-shaped cluster of elongated cells indicate that the dyed cell is clearly within the taste bud.

A TB-2 cell was dye-injected and is shown in Figure 18B, again revealing a cell unmistabably within a taste bud. However, in this section, two cells appear to contain dye. This also might be explained by dye crossing at an electrotonic synapse between two TB-2 cells. For reasons stated below, it is suggested that the TB-1 and the TB-2 cells correspond to the light and dark taste bud cells, respectively, of Farbman and Yonkers (1971).

DISCUSSION

Factors Affecting Cellular Characteristics

Adapting solutions influence the resting potentials of cells and must be considered when other experimental values are compared. 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 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 (see Table 1).

The NS and WS 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 previously categorized on the basis of their responses, similar to the distinction made between TB-1 and TB-2 cells in the experiments reported here. Previous studies may not have tested the cells in a way that was adequate to reveal their differences. 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 used in the previous

study, implying that the adapting solution employed may be 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 (Esakov and Byzov, 1971; Kutyna, 1973) and thus alter subsequent responses. TB-1 cells provide evidence for such a phenomenon, since the membrane potential level greatly affected the responses, especially to the non-salt stimuli. The same TB-1 cell could have quite different response profiles at different membrane potentials; taste cells in other species might react similarly. Expressing the response as a percentage of the resting potential, as done by some other investigators, would not totally correct for this effect. If it were not for the distinctive TB-1 cell response to K-salts, it would be difficult to group these cells into one category on the basis of their response to the other stimuli employed.

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 disc. 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 more primitive, less discriminative manner than taste bud cells. A potential change generated in a large number of SE cells simultaneously might have some effect on the extragemmal nerve fibers that innervate the epithelium around and between buds. Also, the SE cells 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.

Type I familial dysautonomia in humans is associated with a variety of neurological symptoms including the inability to taste. The tongues of these patients lack fungiform or circumvallate papillae and their associated taste buds. However, after administration of metacholine, these patients have the temporary ability to taste and distinguish sapid solutions within the normal concentration ranges (Henkin, 1970). The taste sensations in this case were probably mediated by unmyelinated nerve endings in the

tongue epithelium, and it is conceivable that the non-specialized epithelial cells were responsible for at least part of the input to the nerve fibers in this and the normal situation.

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 aquatic vertebrates responded to chemical stimuli like primitive organs of taste. Considering this similarity between taste and lateral line organs, the enhancement of mechano-sensitivity of the lateral line organs by K^+ (see Literature Review) might be produced by the same cellular mechanism causing the high sensitivity of the TB-1 cells to K-salts.

Response Mechanisms

Several different processes must be responsible for the generation of SE cell responses to chemical stimuli. An outstanding example is the increase in membrane resistance accompanying depolarization to $MgCl_2$ solutions, in contrast to the decrease of resistance during depolarization by other salt solutions, thus implying that different mechanisms produce these potential changes. Both Akaike

et al. (1976) and Ozeki (1971) proposed that the taste cell depolarization produced by NaCl or KCl was caused by an increase in membrane ionic permeability, possibly to Na and Cl. Such a mechanism might be involved in SE cell responses to these and other salts. However, the membrane resistance increase with $MgCl_2$ indicates that this stimulus must act by a different mechanism, possibly by a decrease in permeability to K^+, as proposed by Krnjevic et al. (1976) to explain the depolarization with increased membrane resistance that was observed upon injection of Mg^{++} 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). It was proposed that bitter substances and certain lipid-soluble anesthetics act similarly by penetrating the taste cell membrane and dislocating its crystal lattice, thus altering permeability (Akaike and Sato, 1975). Quinine solutions probably increase SE cell membrane resistance by the same mechanism as in taste bud cells. Acid solutions may also depolarize SE cells by the same mechanism. However, Brown (1972) demonstrated that low pH affected a neuronal membrane potential by changing Cl^- permeability; so perhaps low pH depolarizes SE cells by decreasing membrane permeability to Cl^-. This hypothesis is supported by

the finding that the permeability of striated muscle cell membrane to Cl^- decreased in low pH solutions (e.g., Fink and Lüttgau, 1973).

The hyperpolarization produced by dilute salt solutions and water is probably due to removal of the stimulating cations (e.g., Na) in the salts of the adapting fluid. When the NaCl solution was reapplied after a water stimulus, an additional hyperpolarization was seen, especially in SE cells. Similarly, under water adaptation, NS cells in the frog gave small hyperpolarization before the large depolarization to 0.1 M NaCl. Sato and Beidler (1975) explained the earlier potential change as the result of the Cl^- reaching and inhibiting the cationic sites before the arrival of the excitatory Na^+ because of the higher ionic mobility of the Cl^-. This explanation might apply to the water off-response seen in all three types of cells in the mudpuppy tongue.

Some stimuli evoked similar potential changes in SE and taste bud cells in this study, but the receptor mechanisms for other stimuli varied for different cell types. In particular, $MgCl_2$, H_2SO_4, and QHCl must have depolarized SE and TB-1 cells by different mechanisms, since the membrane resistance changes were different. For the depolarizing action of K-salts on TB-1 cells, the entry of a cation is implied (see Eyzaguirre et al., 1972), since the cells

became positive inside (i.e., response overshot zero potential). The decrease in TB-1 cell membrane resistance indicates that the cation is probably Na^{+} or Ca^{++}, the former being most likely due to its positive equilibrium potential.

The unique hyperpolarizing responses of TB-2 cells to $MgCl_2$, acid and sucrose solutions were associated with very small changes in membrane resistance. One explanation for these potential changes is that large depolarizations in closely neighboring 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 neighboring photoreceptor cell membranes. If the membranes of the TB-2 cells are closely apposed to large areas of membrane of cells that depolarize to $MgCl_2$, acid and sucrose (i.e., TB-1 cells), hyperpolarization of the TB-2 cell could result with minimal membrane resistance change. 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 Electrotonic Coupling in Tongue Epithelium

The occurrence of electrotonic coupling in the tongue mucosa of the mudpuppy is not surprising since it has been demonstrated for every epithelial tissue that has been tested. The coupling ratios found for SE cells were relatively small. However, it should be remembered that the bathing medium lacked Ca^{++}, and lowered extracellular Ca^{++} concentration has been shown to reduce coupling between epithelial cells (see Literature Review). Possibly, enough Ca^{++} had been leached out of the surface tissue by the continuous rinse to reduce or eliminate coupling between these cells, though a few early experiments with a Ringer saline rinse (containing Ca^{++}) gave similar coupling ratios for SE cells.

There are several possible functions for coupling in this tissue. If the SE cells are involved in taste reception, transfer of potentials from cell to cell could be an important effect of the coupling. Referring to coupled neurons in CNS nuclei, Spira and Bennett (1972) stated that a single cell can be strongly excited by the activity of many surrounding cells that are even weakly coupled. Thus, coupling can mediate a positive feedback between cells, thus synchronizing their responses and enhancing their overall sensitivity. Loewenstein et al. (1965) stated that

even the small groups of coupled cells in a sensory epithelium could act as a signal amplifier. In the tongue, a type of peripheral integration could occur, allowing areas of the epithelium involving many cells to act as a unit. Changes in degree of coupling with changing solutions has been reported for several coupled systems, and such changes could be another factor in taste reception if SE cells have an input to taste nerves.

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 (Figure 2) may be due to increased coupling between them, similar to the restoration of coupling by cell repolarization seen in salivary gland cells (Rose and Loewenstein, 1971).

For most epithelial cells, a more likely function of coupling is for metabolic, nutritive, or regulatory interactions of the cells. This possibility is quite plausible for SE and TB-2 cells, but does not exclude an electrical role for the observed communications. For embryos of many species, coupling between cells is extensive until specific differentiation of the cells into various types is required (see Loewenstein, 1973). The lack of electrotonic coupling between TB-1 cells and between taste bud and non-taste bud

cells implies that epithelial cells may become uncoupled as they differentiate into taste bud cells.

Taste Bud Cell Structure and Function

The results of cell dye marking in this study were not conclusive enough to correlate taste bud cell type (as defined for this species by Farbman and Yonkers, 1971) with function. However, there are enough indicators to propose the hypothesis that TB-1 cells and TB-2 cells correspond to light and dark cells, respectively.

One reason for this conclusion is that the dyed TB-1 cells, like light cells, tended to have nuclei deeper in the bud and more centrally located; whereas, TB-2 cell nuclei were generally located toward the periphery of the bud. Also, it was easier to locate, penetrate, and record for longer periods from TB-1 cells than it was for TB-2 cells, which could be due to the more voluminous, regular shape of light cells.

Another morphological finding that supports this hypothesis is that dark cells surround and separate light cells to a large extent, allowing very limited areas of contact between light cells. The larger areas of contact between dark cells would provide greater opportunity for junctions mediating the electrotonic coupling observed between TB-2 cells. Also, the enveloping of TB-1 cells by

TB-2 cells makes it quite possible for a large depolarization in TB-1 cells to hyperpolarize the intervening TB-2 cells.

From cell morphology, Farbman and Yonkers (1971) and Murray (1971) concluded that the dark cells have a supportive, secretory function and the light cells are the taste receptors. The TB-1 cells (the putative light cells) gave large potential changes to many sapid stimuli (e.g., K-salts), as might be expected from a chemoreceptor element. Many of the responses in TB-2 cells (proposed dark cells) resembled the hyperpolarizing secretory potentials recorded by Lundberg (1956) from the cat sublingual gland. This might mean that the TB-2 (dark) cells are stimulated to secrete by certain (possibly irritating) substances. The function of electrotonic coupling between supportive elements (TB-2 cells) could be for nutritive supply, as suggested for coupled glial cells. Contrary or in addition to this supportive role for TB-2 cells, both types of taste bud cells may have an input to the nerve fibers, the distinct differences between many of the responses from the two sources finely differentiating between taste stimuli.

Since response patterns were used in this study to establish cell type, it is possible that a third type of taste bud cell (as yet undyed) gave responses indistinguishable from those of SE cells. This hypothetical taste bud cell would provide another input to the nerve fibers.

SUMMARY AND CONCLUSIONS

1. Surface lingual cells outside of taste buds (i.e., SE cells) gave relatively large responses to chemical stimulation, suggesting a possible role for them in taste reception. Membrane potential and/or resistance changes were especially significant to salt, acid and quinine stimuli, though sucrose occasionally evoked small responses. Chemical responsiveness alone does not necessarily distinguish a cell as a "taste receptor".

2. Different stimuli, even within one stimulus class, may evoke responses in SE cells by different mechanisms as evidenced by membrane resistance changes. Depolarization was accompanied by increased resistance with acids, quinine and $MgCl_2$ and decreased resistance with other salts at concentrations greater than the adapting solution.

3. Cells within the tongue epithelium may be categorized by their different responses to various stimuli. Not only do SE cells differ from taste bud cells, but at least two functional types of taste bud cells exist. TB-1 cells are characterized by large, rapid depolarizations to K-salts; TB-2 cells primarily hyperpolarize to several stimuli (e.g., $MgCl_2$, acids and sugars).

4. Intact lingual circulation is required for distinguishing cell types; taste bud cell responses are especially dependent upon proper circulation. Since SE cells continue to respond for some time without circulation, responses obtained from "taste cells" without proper blood supply may or may not be representative of the normal in vivo situation.

5. The different cell types respond to some of the same sapid stimuli through different membrane mechanisms. Both form and mechanism of responses indicate that taste bud cells are different from the unspecialized epithelial cells.

6. Electrotonic coupling occurs between SE cells and between one type of taste bud cell (TB-2). Coupling does not appear to be present between the other type of taste bud cell (TB-1) nor between cells of different types. Taste response amplification, modulation or interaction may be part of the function of this coupling.

7. There is some morphological and physiological evidence that the TB-1 cells correspond to the light cells and are the taste receptors and that the TB-2 cells correspond to the dark cells, which may have supportive, secretory or (additional) taste receptive function or some combination of these.

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APPENDIX

APPENDIX

Problems of Electrode Artifacts

One of the major problems in obtaining reliable recordings of intracellular potentials arises from the tip potential of the microelectrode. Adrian (1956) investigated these tip potentials and found that electrodes with higher resistances (i.e., smaller tips) tended to have higher tip potentials. Also, the magnitude of a tip potential for each electrode was inversely proportional to the concentration of the medium bathing the electrode tip. Since the amplitude of the recorded membrane potential depends upon the magnitude of the electrode tip potential, intracellular recordings are highly susceptible to artifacts, especially when bathing fluids are changed. This problem is aggravated by the necessity of using small, high resistance electrodes to record from small epithelial cells. Therefore, several steps have been taken to control or check for possible sources of artifacts in this study.

To test the physicochemical effects of changing the bathing medium on the electrode, the same test solutions used during intracellular recording were applied to the electrode prior to several of the experiments.

Such potential and system resistance changes resembled many of those recorded for SE cells. However, there were several specific differences including a difference in time courses between the artifacts and the cellular responses.

Several of these differences were great enough to allow clear distinction between extra- and intracellular recordings from SE cells: (1) The sulfate salts of Na and K both gave hyperpolarizing artifact potential changes (APC) even at 0.3 M; whereas, SE cells gave depolarizations, often to 0.1 M, and always 0.3 M salt solutions. (2) The APC to QHCl was always an abrupt hyperpolarization; cells often depolarized or showed little potential changes, always with a very slow time course. (3) The non-electrolyte sucrose stimulus caused no APC, though these same solutions evoked receptor potentials in many SE cells. (4) The large increase in membrane resistance seen in SE cells to 0.1 M $MgCl_2$ solutions was in direct contrast to the significant decrease of system resistance associated with the APC. (5) SE cells responded with large potential changes that were slow to decay and showed large increases in membrane resistance to H_2SO_4 solutions, but the APC to acid solutions were small (<10 mV), and quick to decay, with slight decrease in system resistance at the higher concentrations. There were other less obvious differences (e.g., hyperpolarizing APC to 0.1 M KCl or NH_4Cl) that added to the evidence that

the APC and the receptor potentials from SE cells were separate events.

Eyzaguirre et al. (1972) reported that tip potential artifacts recorded in free fluid did not contribute significantly to the recorded potentials in cells because they disappeared when the electrode tip was firmly contacting the tongue surface. In the experiments reported here, greatly attenuated or no potential changes were recorded from deep epithelial cells not contacting the external medium. This strongly suggests that the stimulating chemical solutions did not reach the tip of the electrode, in general, once it was within the tongue. Furthermore, subtracting the APC from the receptor potential would not yield an accurate accounting of actual cellular responses since the tip was largely "protected" from the stimulating solutions when inside a cell.

Physically generated potential changes do not show signs of adaptation or fatigue. Yet repeated application of deionized water on SE cells yielded progressively slower responses though the final potential reached was the same. Also, amplitudes and rates of responses to most stimuli decreased during long experiments or after application of a fixative to the responding cell. With prolonged use, tip potentials of electrodes tended to increase with concurrent increase in the APC produced by them.

The fact that several distinct patterns of potential and resistance change were found for different types of cells proves that most of the responses reported here were of biological origin. Differences between APC and TB-1 and TB-2 cell responses were so obvious that no additional proof of their validity is needed. Repeated intracellular dye marking after recording from all three types of cells demonstrated that the electrode tip was indeed inside a reasonably intact cell membrane. In some other reports on taste cells, this marking alone was considered sufficient to discriminate between taste receptor potentials and electrode artifacts (e.g., Sato and Beidler, 1975).