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Regenerative Impulses in Taste Cells

Abstract. *Taste cells and nongustatory epithelial cells in the isolated lingual mucosa from the mud puppy* Necturus maculosus *were impaled with microelectrodes. The taste cells, but not surrounding epithelial cells, were electrically excitable when directly stimulated with current passed through the recording electrode. Action potentials produced by taste cells had both a sodium and a calcium component.*

Little is known about the cellular mechanisms of chemosensory transduction in vertebrate taste buds. Taste cells are small and relatively inaccessible; hence, they have been difficult to study with intracellular microelectrodes. It has been established that taste receptors differentiate from surrounding stratified squamous epithelium (*1*), form synaptic contacts with gustatory nerve fibers (*2*), and convert chemical stimulation by sapid agents into signals that can be transmitted to the central nervous system (*3*). Furthermore, it has been held that taste cells have relatively low resting potentials and linear (ohmic) membrane resistance and that they respond passively, with graded receptor potentials, to chemical stimulation (*3–5*). In this report, I describe a preparation for studying intracellular responses in vertebrate taste cells—the isolated lingual epithelium from the mud puppy *Necturus maculosus*—and provide evidence that these cells have high resting potentials, very high input resistances, and generate sodium and calcium impulses.

I selected *Necturus maculosus* because the taste cells are much larger than those in other vertebrates (*6*). Isolating a thin sheet of lingual epithelium and stretching it flat in a shallow chamber containing Ringer solution allowed individual taste cells and epithelial cells to be distinguished with remarkable clarity, especially with Nomarski optics. The isolated preparation is quite stable, and pharmacological agents added to the chamber gain ready access to taste cells.

Adult mud puppies were kept in well-aerated aquariums filled with recirculated water at about 21°C. Animals were decapitated and pithed, the top of the head and upper jaw were removed, and the lower jaw was pinned firmly on a dissection board, exposing the tongue. A transverse cut through the mucosal epithelium was made with fine scissors, and the epithelial sheet was freed from the underlying connective tissue with blunt dissection. A 1-cm^2 region was removed from the anterior of the tongue, transferred to a recording chamber (*7*), and pinned down, mucosal surface uppermost. During the dissection, the preparation was frequently flushed with cold Ringer solution containing 112 m*M* NaCl, 5 m*M* $CaCl_2$, 3 m*M* KCl, and 3 m*M* Hepes buffer (*p*H 7.2). The Ringer solution in the chamber was identical to that above. Intracellular glass micropipettes were filled with 2.5*M* KCl and had resistances between 50 and 150 megohms.

Stable penetrations with resting potentials up to −90 mV could be obtained reliably from taste cells (*8*). Measurement accuracy was assured by recording resting potentials at the end of an impalement when the microelectrode was abruptly withdrawn from the cell. The input resistance of taste cells was measured by injecting current pulses through the recording electrode (Fig. 1). In a sample of 11 taste cells in which both the resting potential and input resistance were measured, the mean ± standard error (S.E.M.) of the resting potential was −43 ± 6 mV and of the input resistance was 200 ± 30 megohms. These values are likely to be underestimates of the true resting potentials and input resistances, since impaling a cell with a glass micropipette and passing currents across the membrane noticeably injured the taste cells (*9*). Nevertheless, the values for resting potentials and input resistances obtained in these experiments are significantly higher than those reported previously for this species (*5*) or other vertebrate taste cells (*3*).

The most striking finding was that brief depolarizing currents injected into taste cells produced regenerative impulses (Fig. 2) (*10*). These impulses had a relatively low threshold and a brief duration, suggesting that they were mainly sodium action potentials. Occasionally, a slight inflection on the falling phase of the impulses (arrow in Fig. 2A) suggested that there might be other components of the responses, such as calcium currents. Low doses of tetrodotoxin (TTX, 1 μ*M*) rapidly (within 5 to 10 minutes) and reversibly blocked the regenerative impulses. Nevertheless, even in the presence of 1 μ*M* TTX, regenerative responses could be restored if delayed potassium rectification was blocked by

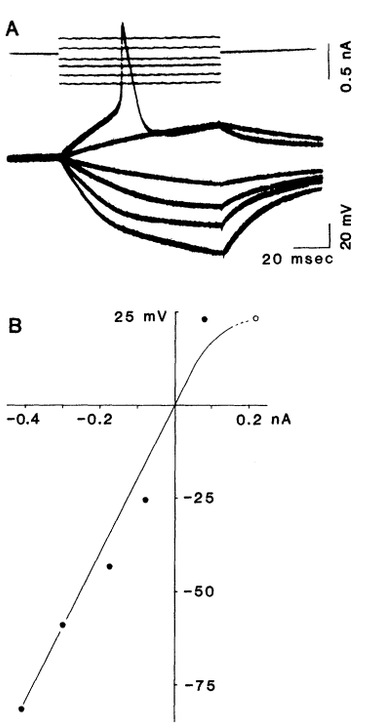

Fig. 1. Current-voltage relation of a taste cell in the mud puppy. (A) Current pulses (upper traces) were passed through the intracellular recording electrode via a bridge circuit. (B) The relation between applied current pulses and changes in membrane potential at the end of the current pulse was plotted and a linear regression line was drawn through the points. The slope of the line yields the input resistance, which in this case was 201 megohms for the linear region (closed circles, r = .977). The open circle in (B) represents the threshold level for impulse generation. The resting potential of this taste cell recorded at the end of the impalement was −73 mV. The constant current pulses did not always produce an exponential rate of change in the membrane voltage, particularly in the hyperpolarizing direction. This finding was made frequently, especially for large hyperpolarizations. Since it was not explained by a fluctuation in the microelectrode resistance when currents were passed, the nonexponential rate of potential change suggests that the passive membrane properties of taste cells from the mud puppy may be more complex than a simple ohmic resistance with parallel capacitance.

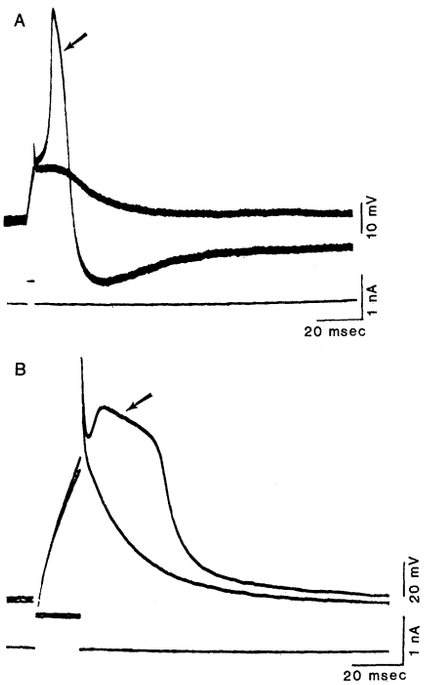

Fig. 2. (A) Intracellular recording of regenerative response in a taste cell from the mud puppy. Two consecutive oscilloscope traces are superimposed. Brief depolarizing current pulses (lower trace) were passed through the recording microelectrode. The membrane depolarizations (upper traces) were just at threshold level. The resting membrane potential was −68 mV. A slight inflection on the falling phase of the impulses (arrow) was frequently observed. (B) Intracellular recording of regenerative response in a taste cell when the bathing solution contained 1 μM TTX and 5 mM TEA. The resting membrane potential was −80 mV. The TEA was added to the bath at least 15 minutes before the responses were recorded. Two consecutive oscilloscope records are superimposed. Brief depolarizing current pulses (lower traces) were injected into the cell through the recording electrode. Membrane potential responses are shown in the upper traces. Impulses generated in the presence of TTX and TEA had pronounced plateaus on their falling phase (arrow).

adding tetraethylammonium bromide (TEA^+, final concentration, 5 mM) (Fig. 2B). Impulses evoked in the presence of TTX and TEA^+ had higher thresholds and pronounced plateaus on their falling phases (arrow in Fig. 2B). These features are indicative of calcium spikes described in other tissues (*11*), and thus these data strongly suggest that taste cells generate impulses having both sodium and calcium components.

Surface epithelial cells in the immediate vicinity of taste buds and elsewhere on the lingual mucosa had markedly lower resting potentials (36 ± 2 mV; N = 30) and did not produce regenerative impulses. I tested whether the absence of regenerative activity might be due to sodium inactivation caused by the low resting potentials, since epithelial cells in some species are capable of generating action potentials (*12*). Brief depolarizing current pulses were superimposed on a d-c hyperpolarization that maintained the epithelial cells at −50 to −60 mV. Alternatively, epithelial cells were hyperpolarized from resting potential with 20- to 100-msec pulses in an attempt to elicit anode break excitation. In no instance were there signs of regenerative excitability in surface epithelial cells.

These data indicate that taste cells in the mud puppy are capable of producing regenerative impulses in response to direct intracellular current injections and that the acquisition of voltage-sensitive sodium and calcium channels is an important step in the differentiation of taste cells from surrounding epithelial cells. Action potentials—probably with a substantial calcium component—and high input resistances have also been observed in amphibian hair cells (*13*). Whether chemical stimulation of taste cells produces receptor potentials that reach threshold and evoke impulses is unknown, but it seems likely that taste cell action potentials, especially their calcium components, play some role in the chemosensory transduction process. For example, influx of calcium could trigger transmitter release from the synaptic foci of taste cells, analogous to the release of acetylcholine from motor nerve terminals (*14*). Regenerative impulses sensitive to TTX and cobalt have been observed in frog taste cells after anode break excitation (*15*). This raises the possibility that sodium and calcium action potentials may be present in taste cells of a number of species and may be a fundamental mechanism in taste transduction (*16*).

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8. Although taste cells in the mud puppy, like those in other vertebrates, can be classified into three or more types based on their morphology, I found no evidence for functional differences in their membrane properties [see (5)]. No attempt was made in the present experiments to differentiate different cell types, such as dark or light taste cells.
9. Prolonged microelectrode impalement, particularly when current was passed through the recording electrode, often produced a visual change in the taste cell. The cell would swell slightly and the cytoplasm became clear. These changes were often accompanied by a decline in the resting potential and input resistance, and in such cases the data were not included. This artifact served a useful purpose, however, since it assured that the microelectrode was inside a taste cell (*10*).
10. Initially there was some concern that the microelectrode had penetrated nerve terminals rather than taste cells. This was ruled out by the following: (i) Taste cells could be identified under the microscope and the microelectrode inserted under direct visual control. (ii) Nerve terminals are only 0.5 to 2 μm in diameter (*6*). (iii) Stable penetrations for periods in excess of 30 minutes could be maintained. (iv) Solutions could be perfused over the isolated tissue while a stable impalement was maintained. (v) Even though some taste cells could be impaled near the base of the taste bud where nerve terminals are abundant, the recordings in these experiments were made from the apical end of the cell in the taste pore where axon terminals do not occur (*6*). (vi) Axons innervating the taste buds were severed very close to the base of the taste buds during the dissection procedures. (vii) Intracellular impalement often produced distinctive changes in the taste cell cytoplasm (*9*).
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