J. Membrane Biol. 95, 265–269 (1987)

The Journal of
Membrane Biology

Action Potentials in Epithelial Taste Receptor Cells Induced by Mucosal Calcium

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Summary. Chemosensory cells in the taste bud of the tongue of *Necturus* generate action potentials in response to electrical stimulation through a microelectrode, as recently described by Roper (*Science*, **220**:1311–1312, 1983). We report that the epithelial receptor cells also respond to 10 mM $CaCl_2$, applied to the mucosal surface, with a depolarization which elicits action potentials when a threshold of −50 mV is reached. Since $CaCl_2$ is one of the taste stimuli in amphibia, the firing of action potentials by chemoreceptor cells may be part of the signal chain in gustatory reception of Ca ions.

Key Words chemoreceptors · taste transduction · secondary sensory cells · epithelial action potentials

Introduction

The relatively large taste receptor cells in the tongue of *Necturus maculosus* can be more easily impaled with microelectrodes than the gustatory cells of other species. As shown by S. Roper, these highly differentiated epithelial cells respond with action potentials to a depolarizing current pulse passed through the recording microelectrode. However: "Whether chemical stimulation of taste cells produces receptor potentials that reach threshold and evoke impulses is unknown . . ." [17].

In similar experiments we stimulated the impaled chemosensory cells from the mucosal side by superfusion with solutions of increased Ca activity. In the frog, Ca ions elicit a gustatory response at concentrations of 0.1 to 50 mM [1, 9, 15; for a review *see* 20]. Exposure to 10 mM $CaCl_2$ depolarized the taste cell of *Necturus* to threshold and evoked action potentials at a typical rate of 0.5/sec.

Materials and Methods

Adult mudpuppies (*Necturus maculosus*) were maintained in aquaria as described by others [8, 17]. Animals were sacrificed by decapitation and pithing and the epithelium of the tongue surface dissected free. Two preparations of 3.1 cm^2 area were usually obtained. A Lucite® ring of 2 cm outer diameter was attached to the mucosal surface with Histoacryl® and placed into a shallow Lucite chamber such that the free interstitial side of the tissue (0.64 cm^2) rested on a slab of Ringer's agar of semi-globular shape (Fig. 1). This arrangement served to keep the impaled cells stationary while the mucosal solution of 0.2 ml volume was changed. The chamber was placed onto the stage of a microscope and the cells viewed with transmitted light, using a 40× water immersion objective or, for impalements, a 10× objective. Cells were impaled from the mucosal side at an angle of 30° using a Leitz manipulator. The experimental table was shock-mounted and shielded by a Faraday cage.

The Ringer's solution on the interstitial side, which connected to the reference electrode (sintered $Ag/AgCl_2$ pellet) was pre-aerated and contained (in mM) NaCl 110, $CaCl_2$ 1, $MgCl_2$ 0.5, K phosphate buffer 3.5 (pH 7.2), pyruvate 5, glucose 5. The mucosal solution was unbuffered 10 mM NaCl (pH 5.7) to which 10 mM $CaCl_2$ (pH 5.75) or other agents were added when indicated. This solution was aerated or oxygenated (100% O_2) prior to use, passed over the mucosal tissue surface by gravity feed at 1 ml/min and removed with a water suction pump. All solutions were used at room temperature.

Microelectrodes were fabricated on an Ensor-type [5] moving-coil puller (Rhema Labortechnik GmbH, Hofheim) from borosilicate glass tubing (Hilgenberg, Malsfeld, FRG) of 1 mm outer and 0.5 mm inner diameter, containing a glass filament. The electrodes were back-filled by capillarity with 3 or 0.5 M KCl, or 3 M K glutamate as indicated. Occasionally 1 mM EGTA was added to the filling solution. With the tip immersed in 10 mM NaCl, the microelectrodes had resistances of 100 to 200 MOhm. The expected change in the electrode tip potential due to movement from 10 mM NaCl (mucosal solution) to 110 mM KCl (cytosol), as calculated with the Henderson equation [3], is in the order of +1 mV.

The 10× preamplifier [4] allowed compensation of tip resistance and input capacitance, and permitted passage of constant current pulses through the recording microelectrode at a gain of 10^{-8} A/V. The stimulator, delivering pulses of 0.5-msec duration, was of our own design. Voltage signals were observed with a storage oscilloscope, recorded on magnetic tape in the FM-mode and played back at reduced speed onto a chart recorder. Low-frequency signals were monitored with a chart recorder during the experiment.

Means are given ± standard deviation (SD).

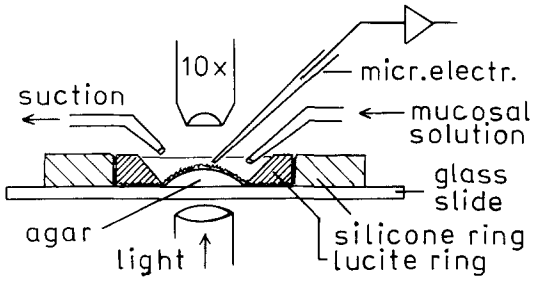

Fig. 1. Schematic cross-section through experimental chamber mounted on the stage of a noninverted microscope

Results

With the light microscope, gustatory eminences containing taste buds [6] were easily recognized in the tongue epithelium. Taste cells were impaled under visual control after passing the electrode tip through a nonsensory surface cell. While we were certain to have recorded from cells within taste buds, we could not distinguish between "dark," "light" and "basal" cells [6]. During impalement of a cell in a bud, the recorded potential jumped to values of −50 to −100 mV. The mean ± SD of 79 cells was −69 ± 11 mV.

Directly following the impalement, or a few seconds later, an action potential was sometimes observed. Subsequently, the potential often declined within 0.3 to 2 min to a less negative steady-state value, or to zero (Fig. 2*A*). During the decline to zero, the voltage noise level was often increased while the potential passed the range −50 to −40 mV. The mean noise threshold of nine cells was −43 ± 11 mV. In six cases a few action potentials (Fig. 2*C*) or a train of increasing repetition frequency and decreasing spike amplitude was superimposed on the declining potential (Fig. 2*B*). The increase in spike frequency with depolarization suggests that the gustatory cells will, in principle, be able to encode stronger depolarizing stimuli by higher rates of firing. The mean threshold for spontaneous action potentials was −53 ± 6 mV.

The spontaneous decline of the membrane potential will be due to cellular damage caused by the microelectrode tip. Efforts were made to pull more slender tips and to use them with different filling solutions. Electrodes having tip resistances of 100 MOhm or less when filled with 3 M KCl caused the potential to decline rapidly, and swelling of the impaled cells was often observed. Filling these tips with 0.5 M KCl improved the recording stability. Tips having 200 MOhm when filled with 3 M KCl did not yield better results when filled with 0.5 M KCl. Replacement of 3 M Cl by glutamate in the filling

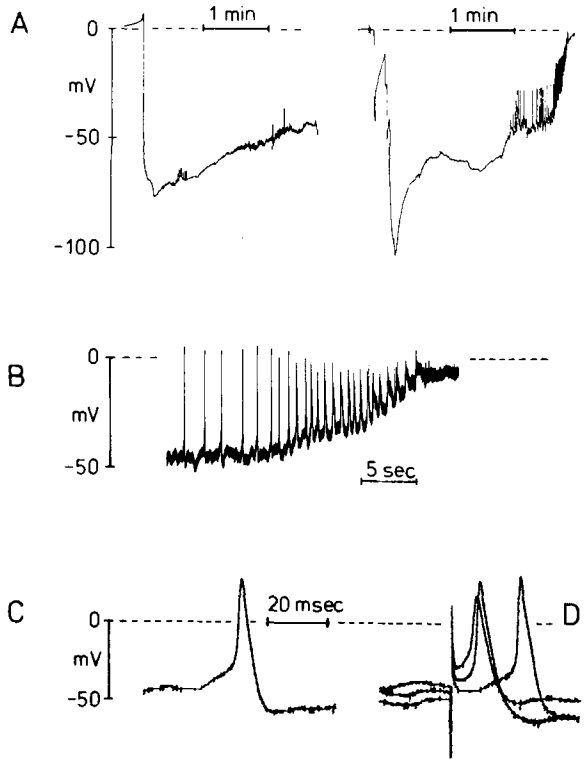

Fig. 2. (*A*) Voltage response of two taste cells to impalement by a microelectrode. On the left, some short deflections at −70 mV were due to electrical stimulation. The noise level increased when the slow depolarization reached −55 mV, and two action potentials were fired. Their amplitudes were not properly represented by the chart recorder. On the right, the voltage first tended to stabilize above threshold, then declined to threshold where a train of action potentials was elicited. Subsequently the depolarization commenced, spike frequency increased and amplitudes diminished. Microelectrode filled with 3 M KCl (left) and 0.5 M KCl (right panel). (*B*) Tape recording of the final spontaneous depolarization shown in panel *A*, right trace. Spike amplitudes are properly represented. (*C*) Action potential of the "fast" type recorded during a period of spontaneous depolarization. (*D*) Action potentials of the "fast" type following a threshold current pulse of 0.5-msec duration (biphasic voltage artifact) passed through the recording electrode

solution or addition of 1 mM EGTA was no improvement.

Some impalements caused a rather slow spontaneous depolarization or allowed stabilization at potentials more negative than −50 mV. The mean stable potential of 19 cells was −61 ± 9 mV. (Means obtained by others with the same preparation were −36, −39 and −43 mV [17, 23]). Cell input resistances in the order of 250 MOhm were then recorded. When stimulated with depolarizing current pulses passed through the recording electrode, these cells often responded with action potentials as shown in Fig. 2*D*. The threshold potential observed

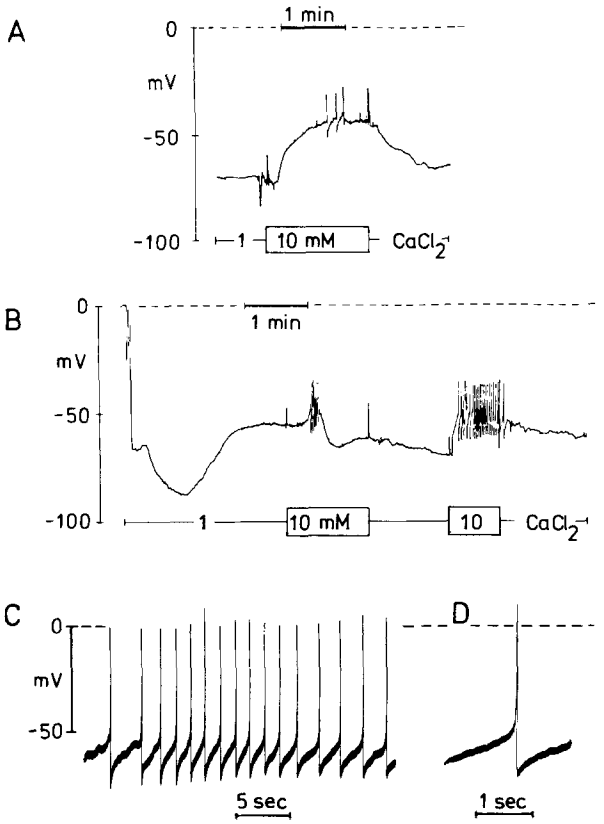

Fig. 3. (*A*, *B*) Depolarization in response to increasing the mucosal Ca concentration from 1 to 10 mM by superfusion. Spike amplitudes are not properly represented. Panel *A* shows three fast spikes, panel *B* on the left a burst of spikes followed by spontaneous repolarization while the exposure to 10 mM Ca continued, and on the right a train of spikes which terminated when the Ca concentration was lowered back to 1 mM. Note the voltage artifacts occurring at the time of solution change. Microelectrode filled with 0.5 M KCl. (*C*) Tape recording of part of the train shown in panel *B*. (*D*) Detail of panel *C*

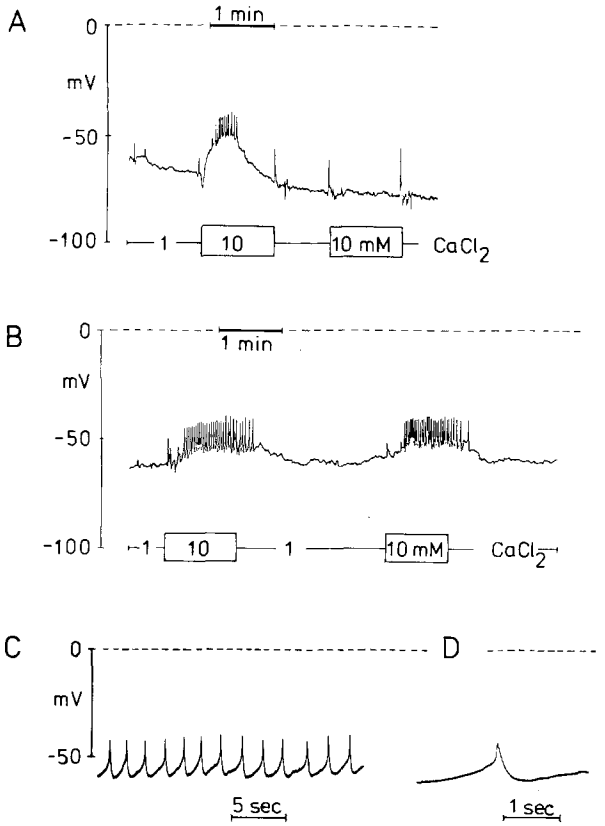

Fig. 4. (*A*, *B*) Responses to 10 mM mucosal Ca of a cell firing "slow" action potentials when depolarized. Spike amplitudes are properly represented. The depolarizing response of panel *A* shows a burst of slow action potentials followed by spontaneous repolarization while the exposure to 10 mM Ca continued. Microelectrode filled with 0.5 M KCl. Note the voltage artifacts occurring at the time of solution change. (*C*) Tape-recorded detail of panel *B*. (*D*) Slow action potential from panel *C* at higher time resolution

with current stimulation appeared to be less negative than −50 mV.

Seventeen cells with stable membrane potentials, and which responded with action potentials to current stimulation, were selected for gustatory stimulation. On increasing the mucosal $CaCl_2$ concentration from 1 to 10 mM by superfusion, a depolarization was recorded (16 cases), the noise level increased (11 cases) and action potentials were fired in eight cases (Fig. 3*A*). Often, but not always, a burst (Fig. 3*B*) or a train of action potentials was observed (Fig. 3*B*,*C*). Within trains the spike frequency was of the order of 0.5/sec.

Different cells generated action potentials of different amplitude. The fastest spikes reached zero mV or overshot zero (Fig. 3*D*), and lasted about 10 msec, as measured at the threshold potential. The falling phase often showed a small shoulder. Each of these fast spikes was followed by a pronounced hyperpolarization and a subsequent semi-exponential depolarization which typically reached the threshold potential (Fig. 3*C*). The mean threshold potential of five cells generating fast Ca-induced action potentials was −50 ± 7 mV. In several instances the action potentials occurred in bursts, i.e. the train terminated during exposure with 10 mM Ca while the potential repolarized spontaneously, sometimes above the starting value (Fig. 3*B*, on the left). This interesting behavior may be indicative of receptor down-regulation.

Three of the cells responsive to mucosal Ca did not generate fast action potentials of high, often overshooting amplitude, but smaller, more drawn-out action potentials, as shown in Fig. 4. (While we are not certain whether these are the Ca action potentials described by others [17], action potentials

of similar slow kinetics and low amplitude are generated for instance by β-cells, where the rising phase is due to activation of inward Ca current and the falling phase due to activation of outward K current [18].) From one such taste cell we could record for one hour, changing the mucosal solution 33 times. In this period the increase in $CaCl_2$ did not in each instance cause depolarization (Fig. 4*A*, on the right), subsequent depolarizations were not always of identical amplitude, and not every depolarization elicited action potentials. The threshold potentials for Ca-induced slow action potentials of the three cells were −55, −55 and −54 mV.

Depolarizations in response to 10 mM $CaCl_2$ were also seen in cells which had membrane potentials less negative than −40 mV. In such cases action potentials were not observed. Depolarizing responses of comparable amplitude were also induced by exposure to 2 mM of mucosal $CoCl_2$ or $NiSO_4$, indicating that the apical receptor mediating the response to these divalent cations is not the classical Ca channel, which does not conduct Co and Ni but is blocked by these ions [7, 12]. Similar depolarizations were observed on mucosal exposure to 200 mM NaCl, indicating that we were recording from taste receptor cells.

Discussion

The method we used for voltage recording from taste cells leaves much to be desired. Even though *Necturus* taste cells [6] are considerably larger than those of gustatory cells in frogs and mammals, and although we used an electrode puller capable of producing very fine tips [5], the impaled cells often gave signs of damage in that their membrane potential decreased spontaneously. While some impaled cells were stable enough to be subjected to further experimentation, their potentials and input resistances were likely to be smaller than those of unimpaled cells.

In the smaller taste cells of frogs the problems are more severe in that cell resistances were found about 10-fold smaller than in *Necturus* and the recorded resting potentials no more negative than −40 mV [1, 15, for a review *see* 21]. In the light of our findings these potentials remained above threshold. Consequently, regenerative potentials were not seen in such experiments, except as anode-break potentials [10] at the termination of hyperpolarizing current pulses.

Despite the well-known difficulties with damage of small cells by impalement, some progress was made with the *Necturus* taste cell in that one mucosal taste agent was found to cause cellular depolarization and firing of a train of action potentials. Their functional significance, in contradistinction to that of mere depolarizing receptor potentials, remains to be determined for these "short" cells. Depolarization is expected to cause release of transmitter from the taste cells [14], and the subsequent excitation of sensory axons of the glossopharyngeal nerve [13, 19, 22]. Some other cells of epithelial origin are also known to generate action potentials. The secretory pancreatic β-cells respond to chemical stimuli with Ca-type action potentials [18], and secretory cells from the anterior pituitary gland with superimposed Na-type and Ca-type spikes [16]. Further experimentation will show whether the action potentials serve for synchronization of neighboring cells coupled by gap junctions.

The choice of $CaCl_2$ among the taste agents was dictated by practical considerations. Other agents (NaCl, sucrose, acids) are effective only at considerably higher concentrations or at low pH [1, 23], where osmotic and other effects disturbing the impalement stability may be expected. Amino acids (threonine, 10 mM, leucine, 10 mM) did not elicit clear responses in trial experiments and the bitter substance quinine will induce a variety of unspecific effects on taste cells [2, 11]. It remains to be seen whether these agents stimulate gustatory cells by mechanisms similar to that of $CaCl_2$.

Our thanks are due to Prof. K. Peper and Mr. H. Ehrler for the loan of a voltage amplifier for these experiments, and to Prof. W. Rummel for the use of the moving-coil electrode puller. Dr. T.D. Plant kindly improved the English style of our manuscript. Support was received from the Humboldt Foundation and from the Deutsche Forschungsgemeinschaft through SFB 246, project C1.

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Received 27 June 1986; revised 8 October 1986