Distribution of Ion Channels on Taste Cells and Its Relationship to Chemosensory Transduction

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Summary. The presence and regional localization of voltage-gated ion channels on taste cells in *Necturus maculosus* were studied. Lingual epithelium was dissected from the animal and placed in a modified Ussing chamber such that individual taste cells could be impaled with intracellular microelectrodes and the chemical environment of the apical and basolateral membranes of cells could be strictly controlled. That is, solutions bathing the the mucosal and serosal surfaces of the epithelium could be exchanged independently and the effects of pharmacological agents could be tested selectively on the apical or basolateral membranes of taste cells. In the presence of amphibian physiological saline, action potentials were elicited by passing brief depolarizing current pulses through the recording electrode. Action potentials provided a convenient assay of voltage-gated ion channels. As in other excitable tissues, blocking current through Na^-, K^+, or Ca^{2+} channels had predictable and consistent effects on the shape and magnitude of the action potential. A series of experiments was conducted in which the shape and duration of regenerative action potentials were monitored when the ionic composition was altered and/or pharmacological blocking agents were added to the mucosal or to the serosal chamber. We have found the following: (i) voltage-gated K^+ channels (delayed rectifier) are found predominately, if not exclusively, on the chemoreceptive apical membrane; (ii) voltage-gated Na^+ and Ca^{2+} channels are found on the apical (chemoreceptive) and basolateral (synaptic) membranes; (iii) there is a K^+ leak channel on the basolateral membrane which appears to vary seasonally in its sensitivity to TEA. The nonuniform distribution of voltage-gated K^+ channels and their predominance on the apical membrane may be important in taste transduction: alterations in apical K^+ conductance may underlie receptor potentials elicited by rapid stimuli.

Key Words taste · ion channels · chemoreception · *Necturus* · sensory transduction · lingual epithelium

Introduction

It is highly probable that chemosensory transduction mechanisms in taste cells involve voltage-gated

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and ligand-gated ion channels that are localized on the apical surface of receptor cells (Teeter et al., 1987; Teeter & Brand, 1987; Roper, 1989a,b). For example, voltage-dependent potassium channels on the apical membrane are closed during chemostimulation by dilute acids, which in humans taste sour. Closure of K^+ channels results in a depolarizing receptor potential (Kinnamon & Roper, 1988a). Cyclic nucleotides may mediate this action on potassium channels in taste cells during stimulation by a variety of chemical stimuli (cf. Avenet, Hofmann & Lindemann, 1988; Tonosaki & Funakoshi, 1988).

A key factor in understanding chemosensory transduction is a knowledge of which ion channels are normally present in taste cells and where they are located on the membrane. It is likely that taste receptor cells, which are specialized epithelial cells, are polarized and that ion channels in the apical membrane differ from those situated on the basolateral membrane, as is the case in other epithelial tissues (Koefoed-Johnsen & Ussing, 1958; cf. reviews by Diamond, 1979; Van Driessche & Zeiske, 1985). Indeed, since taste stimuli act on the apical membrane, one might anticipate that ion channels situated there are the ones most directly involved in chemosensory transduction mechanisms. It is also important to understand the properties and distribution even of ion channels that are not directly affected by taste stimuli since they contribute to the intracellular spread of generator currents.

We have taken advantage of a fundamental biological property of epithelial tissues to study the localization of ion channels in lingual epithelium, particularly in taste cells embedded in this epithelium. This property is the presence of junctional complexes (including zonulae occludens) that seal together the apical regions of epithelial cells, and that create a natural barrier between the mucosal and serosal environments (Staehelin, 1974; Dia-

mond, 1979). By mounting pieces of lingual epithelium between two chambers that can be separately and independently perfused, the extracellular mileau contacting the apical (mucosal) and basolateral (serosal) membranes of taste cells can be rigorously controlled. In this manner, the properties of apical and basolateral ion channels can be studied by ion substitution experiments or by pharmacological manipulations.

This report describes that voltage-dependent Na^+ channels and voltage-dependent Ca^{2+} channels are distributed over the entire membrane of taste cells. In contrast, voltage-dependent K^+ channels (the delayed rectifier) are preferentially situated on the apical membrane and appear in much lower density, if at all, on the basolateral membrane surface. Apical, voltage-dependent K^+ channels are likely to play a prominent role in taste transduction (cf. Kinnamon & Roper, 1988a). Preliminary results of these experiments have been reported in McBride and Roper (1988a,b). Parallel studies, utilizing patch recording techniques on isolated taste cells from *Necturus*, have yielded identical conclusions vis-a-vis K^+ and Na^+ channels (Kinnamon, Dionne & Beam, 1988).

Materials and Methods

Mudpuppies (*Necturus maculosus*) were obtained from commercial suppliers and maintained at 4–10°C. in aquaria containing 2% artificial seawater. Mudpuppies were fed earthworms.

The dissection procedures were similar to those described in Roper (1983) and Kinnamon and Roper (1987). After the lingual epithelium was removed from the tongue with blunt dissection, it was carefully stretched across the bottom half of a modified Ussing chamber which was filled with amphibian saline solution (APS).

Ussing Chamber

We constructed a modified Ussing chamber in which the tissue was mounted horizontally and could be viewed with a compound microscope from the mucosal surface (Fig. 1). The bottom half of the chamber consisted of a plastic plate in which a 0.635-cm diameter hole had been drilled with a shallow O-ring groove milled around it. The floor of the chamber consisted of a glass cover slip. The O-ring served to seal the bottom chamber as well as to provide a means to secure a piece of fine nylon mesh. The nylon mesh supported the lingual epithelium without imposing a diffusion barrier to the medium in the bottom chamber.

Each chamber had four ports: an inlet and outlet port for solutions, a port for a high-impedance voltage-sensing electrode (connected via an agar bridge or electrolyte column to the solution in the chamber), and a port for a low-impedance, current-passing or ground electrode. The lower chamber was a closed system: perfusion was achieved by applying a slight suction adjusted to deliver the solution at about 1 ml/min. The suction also

served to stabilize the tissue during solution changes. Perfusion of the upper chamber was gravity fed at a rate of about 1 ml/min.

The chamber was placed on the stage of a compound microscope and perfusion lines attached to both top and bottom chambers. The tissue was viewed with Nomarski differential interference contrast optics through a 40× water immersion objective and 10× eyepieces. Under these conditions, taste buds can easily be visualized and distinguished from surrounding epithelial cells, and taste cells can be impaled with intracellular micropipettes under direct visual control.

Solutions

Our standard amphibian physiological solution (APS) consisted of the following (in mM): 112 NaCl, 2 KCl, 8 CaCl₂, 5 HEPES buffer (pH 7.2). The calcium concentration was elevated to 8 mM to improve the stability of microelectrode impalements. Tetrodotoxin (TTX) and tetra-ethylammonium bromide (TEA) were obtained from Sigma Chemical Corp.

Electrical Recording

Micropipettes for intracellular recording were filled with 2.5 M KCl and had resistances of 80–200 MΩ. Microelectrodes were inserted into taste cells under visual control at 400×. The reference electrode generally was immersed in the serosal chamber, although using the mucosal chamber as a reference did not alter the findings. A bridge circuit was employed (W.P. Instruments) to pass current into the cell while recording from the same microelectrode. Stable impalements with resting potentials of −50 to −100 mV and input resistances averaging 200 to 400 MΩ could often be maintained in excess of 90 min while continually perfusing both compartments of the Ussing chamber.

The assay for voltage-dependent ion channels we used in this study was the taste cell action potential and the changes in its shape and duration in response to pharmacological agents or ion substitution. Action potentials represent current flux through several different types of ion channels in a specific time- and voltage-dependent sequence. Blocking a specific ion conductance changes the action potential in a predictable fashion, as described in Results. The assay for leak channels consisted of the resting potential and input resistance. These assays primarily give qualitative results and the experimental data did not allow us to make detailed measurements of the densities of ionic channels. Nonetheless, accurate qualitative information can readily be collected by this methodology.

During the course of the experiments, the transepithelial resistance was regularly monitored. This was accomplished by using a 4-electrode voltage/current clamp circuit of our own design and measuring voltage responses in an open-circuit configuration to small current pulses (3 µA/cm²). The viability of the preparation and the ability of the tissue to maintain the isolation between the top and bottom chambers is related to transepithelial resistance. At the beginning of an experiment, typical values of the transepithelial resistance were 1500–2500 Ω · cm². Occasionally, values up to 7000 Ω · cm² were measured. If the initial resistance was less than 1000 Ω · cm², the preparation was discarded. Further, if during the experiment, the transepithelial resistance fell to less than 1000 Ω · cm², the experiment was terminated. Generally, the tissue maintained acceptable specific resistance for more than five hours at room temperature when kept in an open-circuit configuration.
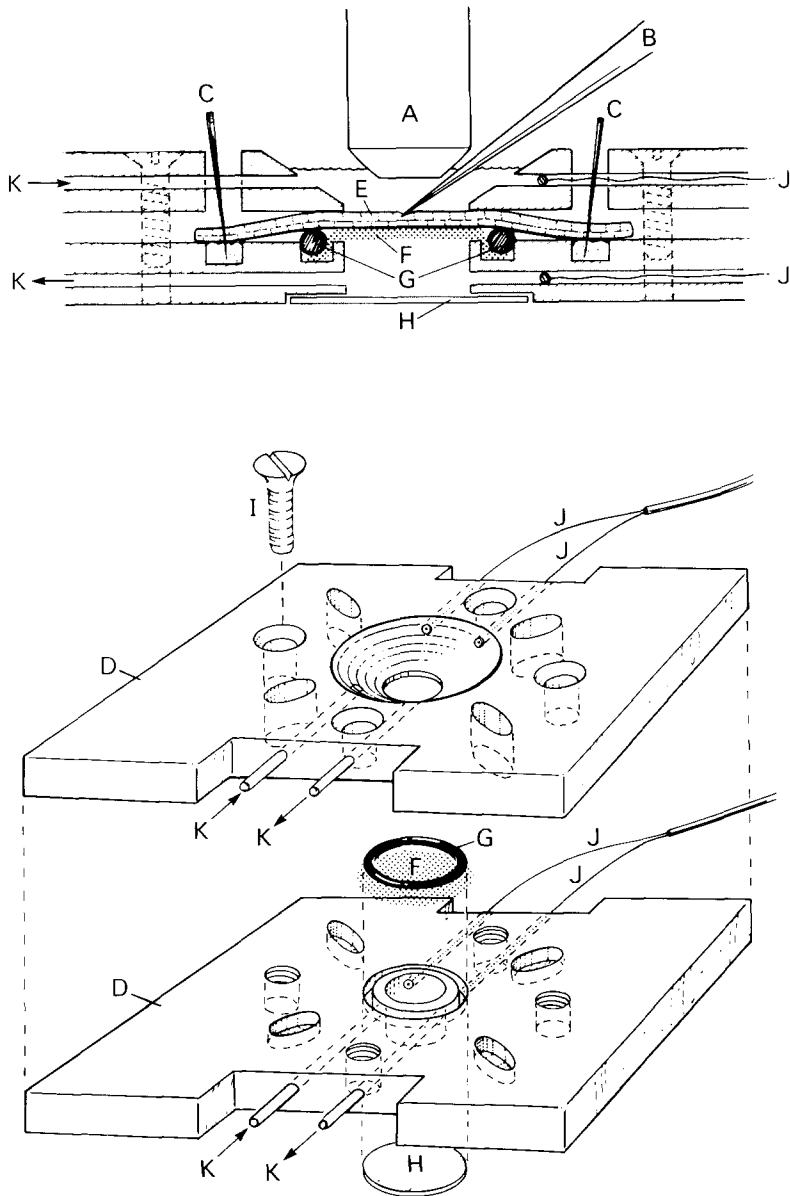
Fig. 1. Diagrammatic representation of the chamber, the tissue, the viewing objective and the placement of the microelectrode. *A*, microscope objective (40× water immersion); *B*, intracellular glass capillary microelectrode; *C*, stainless steel pins; *D*, plexiglass plates; *E*, lingual epithelium from *Necturus;* *F*, nylon mesh; *G*, O-ring; *H*, glass cover slip; *I*, nylon screw; *J*, recording and current-passing electrodes; *K*, perfusion ports

EXPERIMENTAL PROTOCOL

The standard protocol consisted of bathing the top and bottom of the tissue initially in symmetrical solutions of APS. The microelectrode was positioned near a taste bud and inserted into a taste cell until a stable impalement was achieved. The chief criterion for identifying a taste cell was the presence of action potentials in response to brief depolarizing current pulses (Roper, 1983). After a period of about 5 min for stabilization, the solutions perfusing one or both of the chambers were changed from APS to experimental media. The time constant for solution change for the top (mucosal) chamber was more rapid than for the bottom (serosal), but in all cases was less than 1–5 min. To insure that the observations were reversible and that no systematic errors had occurred, test solutions were replaced with APS at the end of the experiment and control recordings rechecked.

Results

Fundamental to the experiments in this study is the ability of the tissue to separate the solution bathing the mucosal surface from that bathing the serosal side. That is, agents applied in one compartment of the Ussing chamber do not readily diffuse into the other compartment. The lingual epithelium of *Necturus* consists of approximately 3–10 strata of cuboidal cells, with the apical regions of the most superficial stratum of cells, including taste cells, sealed together with specialized intercellular junctions, zonulae occludens (Cummings, Delay & Roper, 1987). This serves as a diffusion barrier be-

tween mucosal, or apical membrane, and serosal, or basolateral membrane in the lingual epithelium.

One test of the diffusion barrier is to measure transepithelial resistance, which is related to the junctional resistance created, in part, by zonulae occludens. "Leaky" epithelia have transepithelial resistances <300 $\Omega \cdot cm^2$ while "tight" epithelia have resistances >300 $\Omega \cdot cm^2$ (Diamond, 1979). The mean transepithelial resistance in lingual epithelium from *Necturus* was 2000 ± 1400 $\Omega \cdot cm^2$ ($N = 24$; mean ± SD) when measured in symmetrical solutions of amphibian physiological saline (APS), comparable to that in urinary bladder from this species (Demarest & Finn, 1987a). This classifies *Necturus* lingual epithelium as tight. Furthermore, agents, such as ions, as will be described below, have a pronounced effect on the electrical properties of the taste cells when applied in one chamber but not in the other. These data indicate that there is an effective separation of the solutions bathing the mucosal and serosal surfaces of taste cells in our preparation and that agents can be applied selectively either to the apical or the basolateral membrane. This has been used to advantage to characterize the ionic channels on the apical and basolateral surfaces.

ELECTRICAL PROPERTIES IN AMPHIBIAN PHYSIOLOGICAL SALINE SOLUTION (APS)

The initial condition for all the experiments described in this report is that taste cells were bathed in APS on both apical and basolateral surfaces (symmetrical solutions). Our standard nomenclature for identifying the composition of mucosal and serosal solutions will be: mucosal solution/serosal solution. For example, in the case of symmetrical solutions of amphibian physiological saline, this is identified as APS/APS. Under these conditions, taste cells had resting potentials in excess of −50 mV and generated large action potentials, as described previously (Roper, 1983; Kinnamon & Roper, 1987). The action potentials were of relatively brief duration and often had a pronounced after-polarization lasting several tens of msec (Fig. 2; *cf.* Kinnamon & Roper, 1987). We have previously shown that the fast rising phase of the action potential is due to a TTX-sensitive, inward Na^+ current (Kinnamon & Roper, 1987, 1988b). Potassium, Ca^{2+}, and Ca^{2+}-mediated cation currents contribute to the plateau, or falling phase, and afterpolarization of the action potential in *Necturus* taste cells (Roper, 1983; Kinnamon & Roper, 1988b).
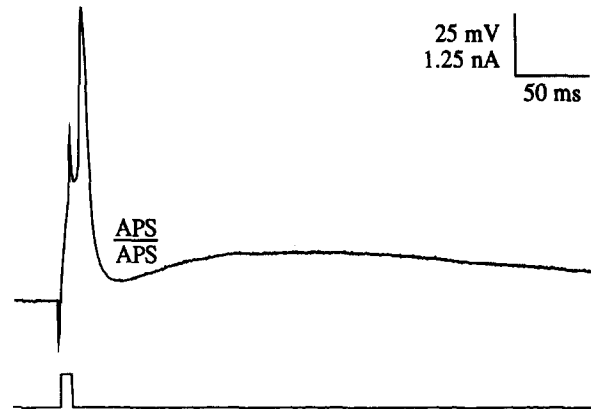

Fig. 2. Action potential elicited from a taste cell in *Necturus*. Apical and basolateral membranes were bathed in amphibian physiological saline (*APS*). Note the prolonged afterpotential following the initial spike. In this and in all subsequent figures, unless noted otherwise, the upper trace shows the membrane potential, the lower trace shows the depolarizing current pulse passed through the intracellular recording electrode, and the resting membrane potential of the taste cell was set to −100 mV by passing a constant DC current through the recording electrode. Mucosal and serosal chambers contained (in mM): NaCl, 112; KCl, 2; $CaCl_2$, 8; HEPES, 5

VOLTAGE-DEPENDENT Na^+ CURRENTS

We discovered that voltage-dependent Na^+ channels were situated on apical and basolateral surfaces. To establish this spatial distribution of voltage-dependent Na^+ channels, we applied 0.1–0.2 μM TTX ($N = 3$ cells); or replaced Na^+ with choline (100% Na^+ replacement; $N = 5$ cells); or replaced Na^+ with N-methyl-D-glucamine (NMDG, 85% Na^+ replacement; $N = 4$ cells) in the serosal chamber (basolateral membrane). In every case, these procedures reversibly abolished or, in the case of NMDG substitution, markedly reduced the amplitude of the action potential, indicating that Na^+ channels are present on the basolateral membrane (Fig. 3).

Similar applications of Na^+ channel blockers or ion substitutions in the mucosal chamber (apical membrane) had little effect on the amplitude of the action potential. This is not surprising considering that the apical membrane constitutes a small percentage of the total cell membrane area; the contribution of apical Na^+ channels to the total Na^+ current in the cell is presumably very small, and eliminating apical Na^+ current would not be expected to alter the action potential recorded from the entire cell. To remedy this, we increased the relative contribution of apical Na^+ channels to the total Na^+ current, i.e., balanced apical and basolateral Na^+ flux. Specifically, we replaced 85% of Na^+ in the serosal chamber (basolateral) with
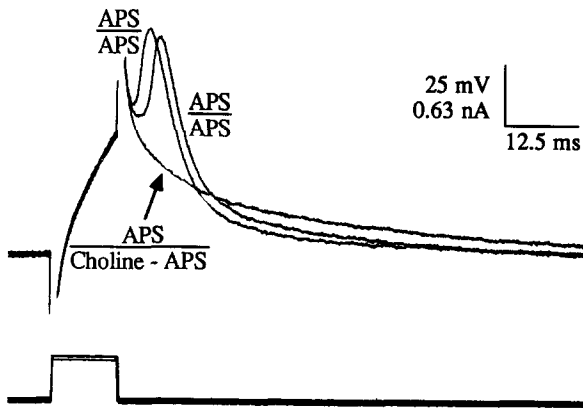
Fig. 3. Voltage-gated Na^+ channels are present in *basolateral* membrane of taste cells in *Necturus*. Effect on the action potential of replacing serosal (basolateral) Na^+ by equimolar choline chloride: the action potential is reversibly eliminated. *APS/APS* (left), action potentials before Na^+ replacement; *APS/Choline-APS*, serosal Na^+ was replaced with choline⁻; *APS/APS* (right), action potential after washing out choline⁺ and restoring Na^+. Similar results were obtained when 0.15 μM TTX was added to the serosal chamber or Na^+ replaced with $NMDG^+$, as described in the text. Mucosal chamber contained (in mM): NaCl, 112; KCl, 2; $CaCl_2$, 8; HEPES, 5. Serosal: NaCl (or choline chloride), 112; KCl, 2; $CaCl_2$, 8; HEPES, 5
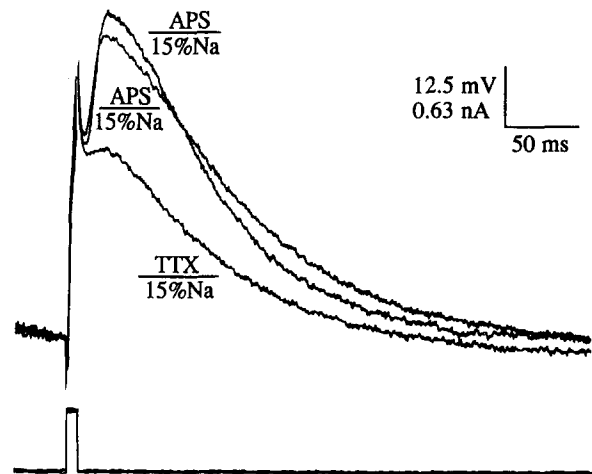

Fig. 4. Voltage-gated Na^+ channels are present in *apical* membrane of taste cells in *Necturus*. The effect of TTX on the apical membrane of taste cells is illustrated. [Na^+] was reduced in the serosal (basolateral) chamber: in all three records 85% of the Na^+ in the serosal chamber was replaced by $NMDG^+$. Mucosal Na^+ was unchanged (i.e., 112 mM). TEA and Cd^{2+} were present in mucosal and serosal solutions to eliminate voltage-gated K^+ and Ca^{2+} channels, respectively. *APS/15% Na* (upper right), action potential before TTX was added; *TTX/15% Na*, action potential when 0.2 μM TTX was added to the mucosal (apical) solution; *APS/15% Na* (lower left), recovery of action potential after washing out TTX. Mucosal chamber (in mM): NaCl, 112; KCl, 2; $CaCl_2$, 1; $MgCl_2$, 7; HEPES, 5; TEA, 5; CdCl₂ 0.1. Serosal: NaCl, 17; NMDG-Cl, 95; KCl, 2; $CaCl_2$, 1; $MgCl_2$, 7; HEPES, 5; TEA, 5; $CdCl_2$, 0.1

NMDG and blocked ionic fluxes through K^+ and Ca^{2+} channels with 8 mM TEA and 0.1 mM Cd^{2+}, respectively. Figure 4 shows the results of one of these experiments (total $N = 39$ experiments). With 15% Na^+ bathing the basolateral membrane and 100% Na^+ bathing the apical membrane, the amplitude of the action potential was markedly reduced. As shown in Fig. 4, applying TTX on the apical membrane under these conditions now significantly and reversibly decreased the amplitude of the action potential. Similar results were obtained when mucosal Na^+ was replaced by NMDG instead of blocking apical Na^+ channels with TTX.

These data indicate that voltage-dependent Na^+ channels are situated on both apical and on basolateral membranes. However, under natural conditions *in situ* when the serosal membrane is surrounded by tissue fluids and the mucosal surface of taste cells is bathed with dilute salts (i.e., saliva), the basolateral membrane alone would be expected to provide sufficient inward Na^+ current to sustain action potentials, should they be important in taste transduction (*cf*. Roper, 1989a,b).

VOLTAGE-DEPENDENT K^+ CURRENTS: THE DELAYED RECTIFIER

Next, we studied the localization of voltage-dependent K^+ channels and found them to be situated

only on the apical ends of taste cells. Potassium current through voltage-dependent K^+ channels (the delayed rectifier) in *Necturus* taste cells is blocked by 5 to 10 mM TEA (Kinnamon & Roper, 1987, 1988a,b). To test the spatial distribution of these channels, we added 8 mM TEA in the mucosal bath (i.e., TEA-APS/APS; $N = 33$ cells). There was a marked increase in amplitude and prolongation of the action potential (Fig. 5), produced by eliminating outward K^+ current and unmasking Ca^{2+} currents (*see below*). These changes were completely and rapidly reversed when TEA was washed out of the mucosal chamber. TEA (8 mM) on the apical membrane also depolarized the resting potential of the taste cells, consistent with the fact that a significant proportion of the *resting* membrane potential is contributed by TEA-sensitive, voltage-dependent K^+ channels (Kinnamon & Roper, 1987).

In contrast, when TEA was applied to the basolateral membrane (APS/TEA-APS), the amplitude and shape of action potentials were for the most part unaltered, even after prolonged exposure to TEA. This was not due to diffusion barriers preventing access of TEA to the tissue since other
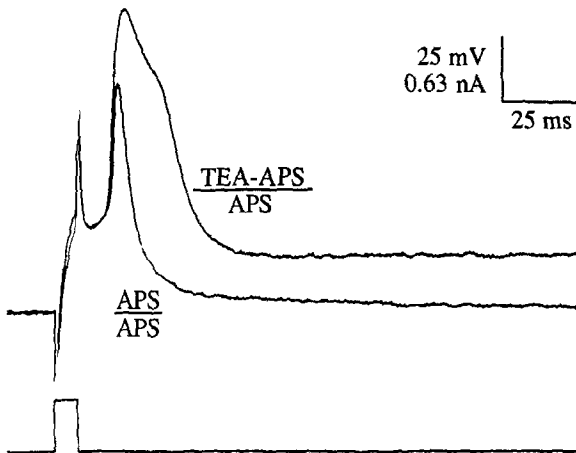
Fig. 5. Voltage-gated K⁻ channels (delayed rectifier) are present in the apical membrane but not in the basolateral membrane of *Necturus* taste cells. Effect of TEA on apical membrane. *APS/ APS*, action potential in symmetrical solutions of APS; *TEA-APS/APS*, action potential in the same cell with 8 mM TEA present in mucosal (apical) chamber. TEA blocks repolarizing K⁺ currents, thereby unmasking an inward Ca²⁺ current and greatly prolonging the action potential. No effect was seen when TEA was added to the basolateral solution. Mucosal chamber (in mM): NaCl, 112; KCl, 2; CaCl₂, 8; HEPES, 5; TEA (when present), 8. Serosal: NaCl, 112; KCl, 2; CaCl₂, 8; HEPES, 5
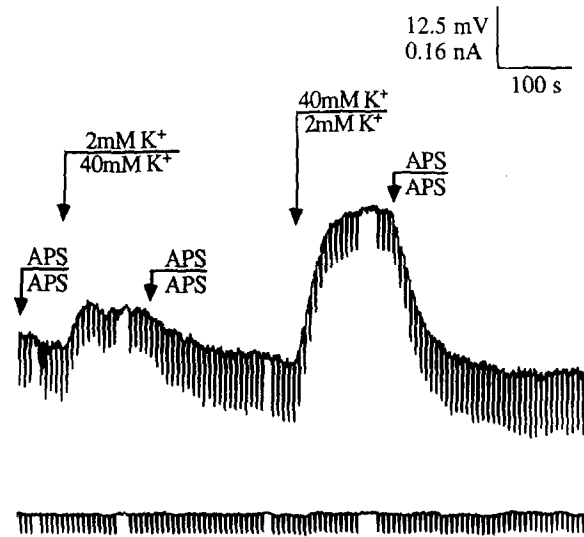
Fig. 6. Passive K⁻ leak conductance occurs in both the apical and basolateral membranes of taste cells in *Necturus*. 40 mM KCl was applied first to the basolateral membrane, then to the apical membrane (arrows). Hyperpolarizing pulses of current (lower trace) were passed through the intracellular micropipette to monitor the input resistance of the cell. No constant hyperpolarzing steady-state current was injected. The initial resting membrane potential was −78 mV. Solutions (in mM): *APS* = NaCl, 112; KCl, 2; CaCl₂, 8; HEPES, 5. 2 *mM K*⁻ = Choline chloride, 112; KCl, 2; MgCl₂, 8; HEPES, 5; CdCl₂, 0.1. *40 mM K*⁻ = choline chloride, 74; KCl, 40; MgCl₂, 8; HEPES, 5; CdCl₂, 0.1

Table 1. Properties of leak K⁺ conductance on apical *vs.* basolateral membrane of taste cells in *Necturus*: responses of cells to 40 mM KCl[a]

N	Initial resting potential (mV)	Depolarization by 40 mM KCl	
		Apical (mV)	Basolateral (mV)
17	−57 ± 14	21 ± 7	
14	−55 ± 9		10 ± 6

[a] Values shown are averages ± SD.

compounds, such as NMDG and TTX reliably and reversibly affected the basolateral membrane. Instead, the data indicate that the delayed rectifier conductance channels are predominantly situated on the apical membrane of taste cells.

K⁺ Channels: Resting Conductance

The spatial distribution of the resting (leak) K⁺ conductance of the taste cell membrane was also investigated. When [K⁺] in the mucosal chamber was elevated to 40 mM (40K-APS/APS), cells were rapidly and reversibly depolarized (Fig. 6; Table 1). Similarly, although to a lesser extent, 40 mM (K⁺) in the serosal chamber depolarized the basolateral membrane (APS/40K-APS). In some experiments,

0.1 mM Cd²⁺ was added with 40 mM K⁺ and Na⁺ replaced with choline to preclude any contributions from voltage-activated Ca²⁺ and Na⁺ fluxes to the resting potential measurements. No significant difference in the results was observed, however.

Experiments were conducted to determine whether the resting leak K⁺ conductance was sensitive to TEA (5–10 mM). Although TEA consistently depolarized the taste cell and increased the input resistance when applied to the *apical* membrane, TEA had variable effects when applied in the serosal (*basolateral*) chamber. In some cases the taste cells were depolarized and the input resistance increased. In other instances, serosal TEA had no significant effect on either the membrane potential or input resistance. This variability seemed to be correlated with the time of year when the experiments were conducted: TEA seemed to have an effect on the basolateral membrane only in the summer months, suggesting a seasonal variation in the pharmacology of the basolateral leak K⁺ conductance. This was not rigorously tested, however. Table 2 summarizes these observations.

Figure 7 shows an experiment where mucosal (apical) K⁺ was elevated to 40 mM, thereby depolarizing the membrane and decreasing the voltage-dependent membrane resistance (i.e., increased K⁺ conductance). When 8 mM TEA was added to this

Table 2. Properties of leak K$^+$ conductance on apical *vs.* basolateral membrane of taste cells in *Necturus:* responses of cells to 8 mM TEA[a]

N	Initial values		Effects of TEA			
	Resting potential (mV)	R_{input} R_{input} (MΩ)	Apical		Basolateral	
			Depolarization (mV)	R_{input} (% control)	Depolarization (mV)	R_{input} (% control)
Summer						
6	−51 ± 4	96 ± 45	27 ± 6	282 ± 43		
6	−58 ± 8	270[b]			21 ± 5	256[b]
Winter						
4	−54 ± 12	127 ± 32	25 ± 4	345 ± 90		
6	−58 ± 14	108 ± 51			−1 ± 4	93 ± 30

[a] Some of these responses varied with the season, as shown above.
[b] Only a single measurement was taken of input resistance in this group.
N = number of cells in the group. R_{input} = input resistance.

(apical) solution, there was an additional depolarization, and the membrane resistance increased. We interpret this sequence as due to an initial change in K$^+$ equilibrium potential, and hence a depolarization of the cell whose membrane is permeable to K$^+$ as [K] is elevated to 40 mM. When TEA is added subsequently, K$^+$ conductance is blocked and the membrane potential is established by its remaining permeability to Na$^+$, Ca^{2+} and Cl$^-$ ions, and by any leak K$^+$ conductance that is TEA insensitive.

Taken together, these data indicate there are passive K$^+$ channels in the basolateral membrane that are not blocked by TEA (at least in winter), and passive, apical K$^+$ channels that are TEA-sensitive. The latter may be identical to the voltage-gated K$^+$ conductance (delayed rectifier) described above that repolarizes the membrane during an action potential. Leak K$^+$ channels that are not voltage dependent and are insensitive to TEA may also occur on the apical membrane. More detailed experiments would be necessary to examine this possibility.

VOLTAGE-DEPENDENT Ca^{2+} CURRENTS

Voltage-dependent Ca^{2+} currents are responsible for the slight plateau or shoulder occasionally observed in action potentials from taste cells bathed in symmetrical solutions of APS (i.e., APS/APS) as demonstrated in Fig. 8. The presence of Ca^{2+} and Ca^{2+}-mediated currents can be revealed by blocking the repolarizing K$^+$ current (delayed rectifier) with TEA. This results in a greatly prolonged action potential (*cf.* Fig. 5). the plateau is directly due to Ca^{2+} entry through voltage-dependent Ca^{2+} channels. The plateau is prolonged by replacing Ca^{2+} with Ba^{2+} (Fig. 9), an ion that is more permeant through
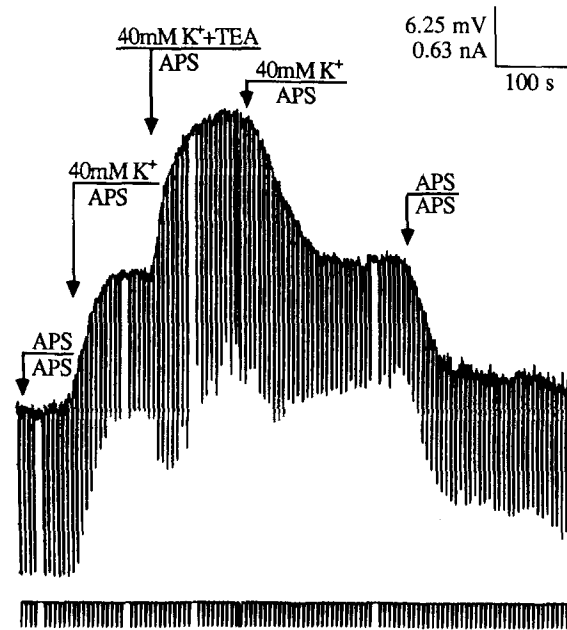

Fig. 7. TEA blocks apical K$^+$ leak conductance in taste cells. 40 mM KCl and 5 mM TEA were added sequentially to the mucosal chamber, shown by the arrows. The upper trace shows the resting potential and input resistance of the taste cell. Constant current pulses (lower trace) were applied to monitor the input resistance. Note the input resistance decreases during the depolarization produced by elevated K$^+$ and then the resistance increases in the presence of TEA, even though the cell becomes more depolarized. Both effects are reversible. The initial resting membrane potential was −45 mV. Solutions (in mM): APS = NaCl, 112; KCl, 2; CaCl$_2$, 8; HEPES, 5. *40 mM K$^+$* = NaCl, 74; KCl, 40; CaCl$_2$, 8; HEPES, 5. *40 mM K$^+$ + TEA* = same as solution 2 with 5 mM TEA added

Ca^{2+} channels in *Necturus* taste cells (*cf.* Kinnamon & Roper, 1988*b*).

To determine the regional distribution of the voltage-dependent Ca^{2+} channels, all voltage-gated
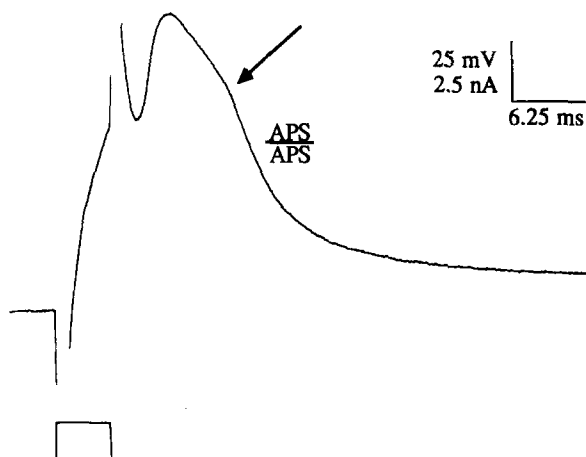
Fig. 8. Action potentials in taste cells often possess a slight inflexion on the repolarizing phase (arrow), indicating a Ca^{2+} component to the action currents. This record was taken when the tissue was bathed in APS (mucosal and serosal)
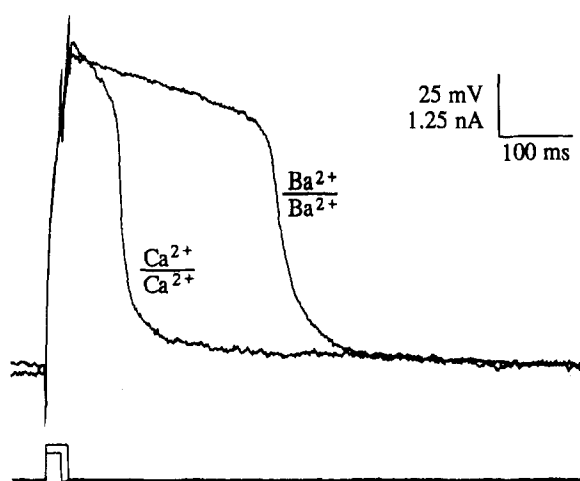

Fig. 9. Taste cells in *Necturus* possess voltage-dependent Ca^{2+} channels. Action potentials (upper traces) were ellicited by brief depolarizing current pulses injected through the recording microelectrode (lower trace). Substituting Ba^{2-} (30 mM) for Ca^{2-} (60 mM) revealed that Ba^{2+} can pass through Ca^{2-} channels, as expected. Note that the duration of the action potential is prolonged in Ba^{2-}, suggesting that intracellular Ba^{2-} does not inactivate voltage-dependent Ca^{2-} channels as rapidly as does Ca^{2-} itself. Solutions (in mM): Ca^{2+} = NaCl, 34; KCl, 2; $CaCl_2$, 60; HEPES, 5; TTX, 0.001; TEA, 8. Ba^{2-} = NaCl, 34; KCl, 2; $BaCl_2$, 30; HEPES, 5; TTX, 0.001; TEA, 8

Fig. 10. Voltage-dependent Ca^{2+} channels are present in the basolateral membrane of taste cells in *Necturus*. *APS/APS*, action potential when the tissue is bathed in symmetrical solutions of APS. Cd^{2+} + *TTX* + *TEA/NMDG-APS*, action potential across the basolateral membrane recorded in the same cell when serosal Na^+ was replaced by $NMDG^+$. TEA, Cd^{2+}, and TTX were added to the mucosal chamber to eliminate K^+, Ca^{2-}, and Na^+ fluxes across the apical membrane. Solutions (in mM): *APS* = NaCl, 112; KCl, 2; $CaCl_2$, 8; HEPES, 5. Cd^{2+} + *TTX* + *TEA* = NaCl, 112; KCl, 2; $CaCl_2$, 1; $MgCl_2$, 7; HEPES, 5; $CdCl_2$, 0.1; TTX, 0.0005; TEA, 8. *NMDG-APS* = NMDG-Cl, 112; KCl, 2; $CaCl_2$; $CaCl_2$, 8; HEPES, 5

results of these experiments when testing for the presence of voltage-dependent Ca^{2+} channels on the basolateral membrane. One trace (APS/APS) is the action potential in symmetrical APS solutions. The other is an action potential generated when all voltage-gated currents are eliminated in the apical membrane and only Ca^{2+} current remains unblocked in the basolateral membrane. The large regenerative response represents a Ca^{2+} action potential across the basolateral membrane.

The task of determining whether voltage-gated Ca^{2+} channels are present in the apical membrane presented the same problem as establishing that inward Na^+ current existed in the apical membrane (above). When voltage-gated channels in the basolateral membrane were blocked and current was allowed to pass through Ca^{2+} channels in the relatively limited area represented by the apical membrane, it was rarely possible to ellicit a *bona fide* regenerative response. Figure 11 shows results of one successful example when Ca^{2+} in the mucosal (apical) chamber was replaced with 15 mM Ba^{2+} (Na^+ was decreased to 79 mM to maintain isotonicity) and all inward current in the basolateral membrane blocked. Similarly, when we increased Ca^{2+} in the mucosal chamber from 8 to 30 mM, we were able to evoke weak regenerative Ca^{2+} action potentials across the apical membrane (3 out of 3 cells).

channels on one region of the taste cell were blocked with TTX (or NMDG substitution) to eliminate inward Na^+ current, TEA to eliminate outward K^+ current, and 1 mM Ca^{2+} plus 0.1 mM Cd^{2+} to eliminate Ca^{2+} current. In the opposite chamber, all voltage-dependent conductances were similarly eliminated *except* for Ca^{2+}. Figure 10 shows the
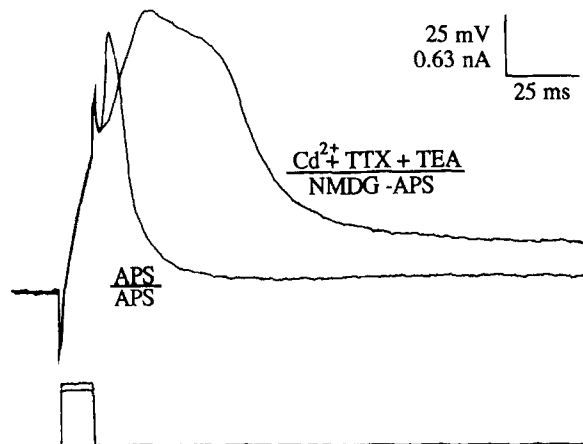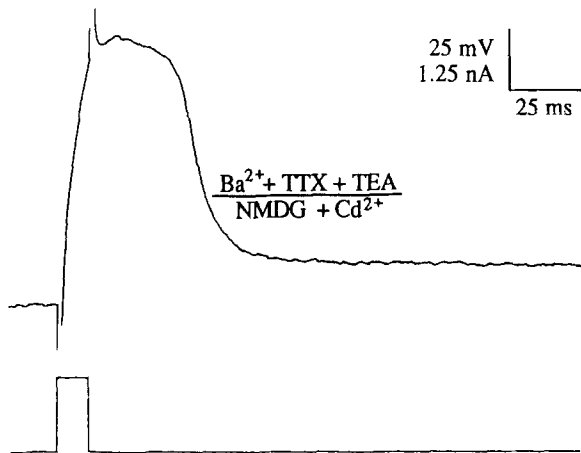
Fig. 11. Voltage-dependent Ca^{2+} channels are present in the apical membrane. Substituting Ba^{2+} for Ca^{2+} in the mucosal chamber and blocking voltage-dependent conductances in the basolateral membrane allows a regenerative impulse to be generated across the apical membrane, representing influx of Ba^{2+} through Ca^{2+} channels. Mucosal solution contained (in mM): NaCl, 79; KCl, 2; $BaCl_2$, 15; HEPES, 5; TTX, 0.0005; TEA, 8. Serosal solution: NMDG-Cl, 112; KCl, 2; $CaCl_2$, 1; $MgCl_2$, 7; HEPES, 5; $CdCl_2$, 0.1

Thus, both apical and basolateral membranes of taste cells possessed voltage-gated Ca^{2+} channels.

Discussion

These findings indicate that some, but not all ionic channels embedded in the membrane of taste cells are differentially distributed between the apical and basolateral regions. Specifically, passive leak K^+ channels are located on both the basolateral and the apical membranes, with the leak K^+ channels on the basolateral membrane being insensitive to TEA (at least, in winter). Voltage-gated Ca^{2+} and Na^+ channels occur on apical and basolateral regions. In contrast, voltage-dependent, TEA-blockable K^+ channels (delayed rectifier) are preferentially situated on the apical membrane. These data, summarized in Table 3, confirm and extend the findings of Kinnamon et al. (1988), who studied the distribution of Na^+ and K^+ channels on isolated Necturus taste cells with extracellular patch-recording technology.

A common property of epithelial tissues in general is that the apical and basolateral membranes of the cells comprising the epithelium are different from each other (Diamond, 1979; Van Driessche & Zeiske, 1985). Each membrane is specialized with regard to the particular role it contributes to the overall functioning of the cell or epithelium. Recently, a number of workers have reported many

Table 3. Summary of ionic channel distribution on taste cells from *Necturus*

	Apical (chemosensitive membrane)	Basolateral (includes synaptic membrane)
Voltage-gated:		
K^+ channels	X	—
Na^+ channels	X	X
Ca^{2+} channels	X	X
Leak channels:		
K^+	X[a]	X[b]

[a] These channels may be identical to or include the voltage-gated, TEA-sensitive K conductance that is found on the apical membrane since the latter is noninactivating and is partially open at rest (Kinnamon & Roper, 1987).
[b] These channels may include inwardly rectifying K channels (cf. Kinnamon & Roper, 1988b).

examples of assymetrical distributions of ion channels in a variety of epithelial tissues (e.g., Sandle & McGlone, 1987; Hunter et al., 1987; Udea, Loo & Sachs, 1987; Hunter, Kawahara & Giebisch, 1988).

Taste receptor cells are modified epithelial cells embedded in lingual epithelium. As such, it is not surprising that the apical and basolateral membrane properties of taste cells differ. The apical membrane of taste cells mediates chemosensory reception and is exposed to varying concentrations of chemical stimuli. The basolateral membrane of the same cell, on the other hand, is not directly involved in the initial step of chemoreception and is exposed to a more constant ionic environment. The basolateral membrane is the site of synapses with afferent nerve fibers and is the site of information transfer, presumably through the release of neurotransmitters. Thus, in taste cells there is a clear functional specialization of apical and basolateral membranes and presumably the difference between ionic conductances of apical and basolateral regions reflects these specializations. In particular, the assymetrical distribution of K^+ channels reported here and by Kinnamon, Dionne and Beam (1988) is significant because the initial events of chemosensory transduction occur at the apical membrane and some chemical stimuli, for example acids (which are sour to humans), have been postulated to depolarize taste cells by blocking voltage-gated K^+ channels (Kinnamon & Roper, 1988a). Blocking apical K^+ channels will produce depolarizing receptor potentials across the basolateral (synaptic) membrane by virtue of a loop current across the basolateral membrane, through paracellular shunts, and back to the apical membrane. That is, electrical events in the

apical and basolateral membranes are coupled via current flow between the two regions.

Kinnamon and Roper (1987) concluded that a major portion of the resting membrane conductance of *Necturus* taste cells was largely a voltage-sensitive K^+ conductance. This finding was based on tissues that were bathed in symmetrical solutions of APS with no attempt to isolate the mucosal from serosal membranes. The present experiments clarify this conclusion: both apical and basolateral membranes possess a resting K^+ conductance. The apical voltage-gated conductance is always blocked by TEA, while the TEA sensitivity of the basolateral K^+ conductance seems to vary from summer to winter. The seasonal variation of the TEA-sensitive channels on the basolateral membrane was unexpected but not unprecedented. For example, the urinary bladder of *Necturus* also exhibits seasonal variations (Karnaky et al., 1984; Demarest & Finn, 1987a).

Other investigators have studied the biophysical properties of the apical and basolateral membranes of taste cells *in situ*, particularly in frogs (Kashiwayanagi, Miyake & Kurihara, 1983; Sato et al., 1984; Okada, Miyamoto & Sato, 1986; Herness, 1987). To study properties of the basolateral membrane, the contents of the interstitial fluid surrounding the taste cell were changed either by perfusing the lingual artery or by prolonged perfusion of the surface of the tongue, relying on diffusion across the epithelium to replace the interstitial mileau. For technical reasons, these methods precluded intracellular recordings on a single cell during the perfusion. Kashiwayanagi et al. (1983) described the presence of voltage-gated Na^+ and Ca^{2+} channels in frog taste cells but did not specifically address the question of their distribution. Sato et al. (1984) and Okada et al. (1986) studied passive properties of frog taste cells and reported that apical and basolateral membranes have passive K^+ conductive pathways. Okada et al. (1986) further probed this K^+ conductance and found that TEA affects neither membrane. Using the whole cell patch-clamp technique on dissociated frog cells, Avenet and Lindemann (1987a) also found that the voltage-dependent K^+ conductance (which they did not localize to apical or basolateral membranes) is TEA insensitive. Thus, the results in *Necturus* differ from those in frog regarding the TEA sensitivity of K^+ channels. Avenet and Lindemann (1987b) also used a related approach (i.e., separating apical from basolateral chambers) to study the chemosensitivity specifically of apical membrane of frog taste cells to Ca^{2+}. These investigators, too, recorded action potentials from taste cells, presumably generated by voltage-gated channels similar to those reported in this study.

No attempt in this study was made to distinguish between the different types of putative chemoreceptor cells in *Necturus*, that is, dark and light cells (Farbman & Yonkers, 1971; Cummings et al., 1987; Delay & Roper, 1988). On the other hand, there were no systematic differences in the results that would suggest two or more populations of cells were involved. Furthermore, Yang and Roper (1987) injected Lucifer yellow into *Necturus* taste cells and found that both light and dark cells generated action potentials. Thus, we conclude that both dark and light cells share the features reported in this study.

A cell type which was not investigated, however, was the basal cell. This cell is small, spherical, and has no marked polarity with regard to morphological features (Farbman & Yonkers, 1971; Delay & Roper, 1988). Since basal cells reside deep in the taste bud and do not extend processes to the taste pore, it is not feasible to impale them with microelectrodes inserted from the mucosal surface. It is believed that the basal cell is a precursor to the dark and light cells, at least in some species (e.g., Delay, Kinnamon & Roper, 1986), and thus it will be of interest to determine what sorts of ionic conductances this cell possesses. It is likely that differentiation of taste receptor cells from basal cells, if this occurs in *Necturus*, involves not only morphological changes, but also involves the acquisition of apical chemosensory receptors and the full complement of ion channels described in this report.

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