Localization of Amiloride-Sensitive Sodium Current and Voltage-Gated Calcium Currents in Rat Fungiform Taste Cells

Albertino Bigiani and Valeria Cuoghi
Dipartimento di Scienze Biomediche, Università di Modena e Reggio Emilia, Modena, Italy

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Bigiani A, Cuoghi V. Localization of amiloride-sensitive sodium current and voltage-gated calcium currents in rat fungiform taste cells. J Neurophysiol 98: 2483–2487, 2007. First published August 8, 2007; doi:10.1152/jn.00716.2007. Recent studies have shown that taste cells transducing bitter, sweet, and umami stimuli do not possess high-threshold voltage-gated calcium channels required for synaptic transmission at conventional synapses, suggesting some sort of signal processing inside taste buds prior to the activation of nerve endings. To evaluate whether this is a general paradigm for the physiology of taste reception, we studied the transduction pathway underlying the detection of sodium ions (salty stimulus). In laboratory rodents, Na⁺ is thought to be transduced, at least in part, through amiloride-sensitive sodium channels (ASSCs). Therefore we used the patch-clamp techniques to analyze the occurrence pattern of amiloride-sensitive sodium currents and voltage-gated calcium currents (both low-voltage-activated T-type current and high-voltage-activated L-type current) among taste cells in rat fungiform papillae. Because taste cells turnover, we focused our attention on cells possessing large voltage-gated sodium currents, a sign of “mature” cells. We found that cells expressing functional ASSCs either did not possess any calcium currents or exhibited only T-type calcium currents, which is believed to play a role in repetitive firing. On the contrary, cells lacking functional ASSCs were endowed with L-type calcium currents, which are thought to mediate calcium influx required for neurotransmitter exocytosis. Therefore our data suggest that sodium-detecting cells are unlikely to use conventional synaptic communication to transfer taste information to nerve endings. Our findings on sodium taste detection support the recent model of taste transduction, involving separate groups of taste cells: chemosensitive cells and cells forming conventional synapses.

INTRODUCTION

Taste recognition of sodium ions in food is a key factor for electrolyte homeostasis in terrestrial vertebrates. In humans, Na⁺ elicits a prototypic sensation called salty taste (Mattes 1997; Schiffman and Erickson 1971). The transduction mechanisms by which taste sensory cells transform an increase in the oral concentration of Na⁺ into electrical signals traveling through gustatory nerves have been investigated in great details in laboratory rodents. The available data suggest that one possible pathway involves the direct entry of food Na⁺ into taste cells via open amiloride-sensitive sodium channels (ASSCs) occurring at apical membrane, which is bathed by saliva (reviewed by Lindemann 1996). This Na⁺ influx produces membrane depolarization (receptor potential) followed by action potential firing (Avenet and Lindemann 1991; Furue and Yoshii 1997; Gilbertson et al. 1992, 2001). The final events in the transduction cascade, namely how taste cells relay “salt” information to nerve endings, are less understood. It is hypothesized that action potential firing in taste cells opens voltage-gated calcium channels and that the influx of calcium ions would eventually trigger neurotransmitter release and activation of nerve endings (Lindemann 1996).

Recent studies have shown that taste cells responsible for the transduction of bitter, sweet, and umami stimuli are unlikely to communicate with nerve fibers by using conventional chemical synapses. In mouse taste buds, two distinct functional classes of taste cells exist: the “receptor” cells and “synapse-forming” cells (Clapp et al. 2006; DeFazio et al. 2006; Medler et al. 2003). Receptor cells are chemosensitive, as indicated by the expression of taste receptors T1R and T2R and G protein gustducin, but lack high-threshold voltage-gated Ca²⁺ channels and synaptosomal-associated protein 25 (SNAP25), which are normally required for synaptic transmission at conventional synapses. The molecular machinery required for calcium-triggered transmitter exocytosis is expressed by another, separate subset of taste cells; however, these synapse-forming cells are not chemosensitive.

In this paper, we have investigated whether the model predicting separate functional classes of “receptor” cells and “presynaptic” cells applies also to the transduction underlying sodium detection. To this aim, we have used electrophysiological techniques (patch-clamp recording) to study the occurrence of ASSCs and voltage-gated calcium channels in taste cells and to establish whether the two classes of ion channels co-localize. Functional properties of ASSC and voltage-gated calcium currents have been studied in detail in rat fungiform papillae (Avenet and Lindemann 1991; Béhé et al. 1990; Doolin and Gilbertson 1996; Furue and Yoshii 1997; Kossel et al. 1997). For this reason, we analyzed the electrophysiological properties of single taste cells in taste buds isolated from rat fungiform papillae.

METHODS

Experiments were performed in compliance with the Italian law on animal care No. 116/1992 and in accordance with the European Community Council Directive (EEC/609/86). All efforts were made to reduce both animal suffering and the number of animals used.

Isolation of rat fungiform taste buds

Adult male Sprague-Dawley rats were used in this study. Our procedure to isolate taste buds from the fungiform papillae closely followed published protocols (e.g., Doolin and Gilbertson 1996;
Kossel et al. 1997). Taste buds were plated on the bottom of a chamber consisting of a standard glass slide onto which a silicon ring 1–2 mm thick and 15 mm ID was pressed. The glass slide was precoated with Cell-Tak (~3 μg/cm²; Becton Dickinson) to improve adherence of isolated taste buds to the bottom of the chamber. The chamber was placed on the stage of an upright Olympus microscope (model BHWI), and taste buds were viewed with Nomarski optics. During the experiments, isolated taste buds were continuously perfused with Tyrode solution [containing (in mM) 140 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 10 glucose, 10 sodium pyruvate, and 10 HEPES; pH 7.4] by means of a gravity-driven system.

Electrophysiology

Whole cell patch-clamp recordings were made from cells in isolated taste buds as described previously (e.g., Doolin and Gilbertson 1996; Kossel et al. 1997). Recording pipettes were made from soda lime glass capillaries (Baxter Scientific Products, McGaw Park, IL) on a two-stage vertical puller (Model No. PP-830, Narishige, Tokyo, Japan). Typical pipette resistances were 2–4 MΩ when filled with a standard pipette solution containing (in mM) 120 KCl, 1 CaCl₂, 2 MgCl₂, 10 HEPES, 11 EGTA, and 2 ATP, pH 7.2 adjusted with KOH. In some experiments, CsCl replaced KCl to block K⁺ currents and reveal the presence of Ca²⁺ currents.

Leakage and capacitive currents were not subtracted from currents under voltage clamp, and all voltages have been corrected for liquid junction potential measured between pipette solution and Tyrode (bath) solution (Neher 1992). Input resistance (R_{input}) of taste cells was measured as the slope of the linear current-voltage (I-V) relationship around ~80 mV.

The presence of functional amiloride-sensitive sodium channels in the cell membrane of taste cells was monitored by studying the effect of amiloride (1 μM) on the whole cell current recorded at a given holding potential (e.g., Doolin and Gilbertson 1996; Gilbertson et al. 1993).

Currents through voltage-gated Ca²⁺ channels were recorded by using Ba²⁺ as main extracellular cation (Béhé et al. 1990; Furue and Yoshii 1997; Medler et al. 2003). To this end, normal Tyrode solution was modified as follows (in mM): 100 BaCl₂, 10 glucose, 10 HEPES, and 1 pyruvic acid, pH to 7.4 with Ba(OH)₂.

RESULTS

Taste cells are in continuous turnover (Beidler and Smallman 1965; Farbman 1980; Hendricks et al. 2004), and the expression of ion channels may undergo maturation processes (Bigiani et al. 2002; Mackay-Sim et al. 1996). An electrophysiological hallmark of “mature” taste cells is the ability to fire action potentials due to the presence of large voltage-gated sodium currents, I_{Na} (Bigiani et al. 2002; Mackay-Sim et al. 1996). Therefore in this study, we analyzed amiloride-sensitive sodium currents and voltage-gated calcium currents in taste cells endowed with large (>500 pA) I_{Na}. Figure 1, A and B, shows examples of membrane currents recorded from two different taste cells maintained in normal Tyrode solution. Although both cells exhibited similar I_{Na} (downward deflections in the current records), they differed markedly for the amplitude of the outward currents (upward deflections in the current records). These currents were blocked by TEA, a general potassium channel blocker, indicating that they were mediated by K⁺ (data not shown). By recording from a total of 54 cells, we were able to verify that mature taste cells in rat fungiform papillae could be subdivided into two distinct groups: cells endowed with potassium currents of low-amplitude (on average, ~500 pA at +50 mV), named hereafter as A cells (Fig. 1A); and cells with potassium current of large amplitude (on average, ~4,000 pA at +50 mV), named hereafter as B cells (Fig. 1B).

A cells were further distinguished from B cells by the low value of input resistance (Fig. 1C, left) and by a low negative value of their zero-current potential (V₀, Fig. 1C, right), a rough estimation of the cell’s resting potential in whole cell recordings (Bigiani et al. 1996). These findings were consistent with the presence of open channels in the membrane of A cells.

The occurrence of ASSCs in the cell membrane of taste cells was shown by studying the effect of amiloride, a diuretic drug, on the whole cell (WC) current recorded at a negative holding potential (e.g., Doolin and Gilbertson 1996). We used amiloride concentration of 1–50 μM (IC₅₀ value for amiloride effect in rat taste cells is ~0.1 μM; e.g., Doolin and Gilbertson 1996; Kossel et al. 1997). Figure 2 shows an example of the effect of amiloride on the WC current. Amiloride caused a reduction in the stationary inward current (Iᵢ) due to block of ASSCs: we will refer to this current reduction as the response to amiloride. The analysis of the response to amiloride revealed that only A cells exhibited large amiloride-blockable currents (Fig. 2A). B cells, on the contrary, were unresponsive or slightly sensitive to amiloride (Fig. 2A). Therefore functional ASSCs appeared to be segregated in a well-defined electrophysiological subgroup of taste cells (Fig. 2B).

After recording the amiloride responses, in the same cells we focused on the occurrence of voltage-gated calcium currents. To study current through calcium channels with high resolution, we replaced the Na⁺ of the Tyrode solution with 100 mM Ba²⁺. Ba²⁺ is known to pass calcium channels better than Ca²⁺ and to reduce outward potassium currents (Bean 1992). Barium currents in taste cells were then elicited by step depolarization from a holding potential of ~90 mV. In agreement with previous studies (Béhé et al. 1990; Furue and Yoshii 1997), we found that rat fungiform taste cells exhibited two types of “calcium” currents: a transient (T-type) current and a sustained (L-type) current. Figure 3 shows sample records of these calcium currents. As described earlier (Béhé et al. 1990; Furue and Yoshii 1997), the T-type activated at less depolarization from a holding potential of ~100 mV (peak value at ~20 mV <100 pA), whereas L-type current could be >1 nA in our recording conditions. Both currents were blocked by 0.5 mM Cd²⁺ (Fig. 3, A and B), an inorganic blocker of calcium channels (Bean 1992). Note that the two calcium current components can be readily distinguished on the basis of their different voltage dependence: T-current activates at about ~50 mV and peaks at about ~20 mV; L-current, on the contrary, activates at about ~10 mV and peaks at ~20 mV (Béhé et al. 1990; Furue and Yoshii 1997). This difference in the voltage dependence of T- and L-components allowed us to distinguish between them when they occurred in the same cell (Fig. 3B).

As to the occurrence of the calcium current types among the two subsets of fungiform taste cells, we found a distinct pattern of functional expression (Fig. 3C). Cells expressing ASSCs (A cells) either did not possess any calcium currents (17 of 33 cells) or exhibited only T-type calcium currents (16 of 33...
cells). On the contrary, all B cells tested \((n = 12)\) were endowed with both T- and L-type calcium currents.

**DISCUSSION**

Our electrophysiological study indicates that in rat fungiform papillae, amiloride-sensitive sodium currents and voltage-gated calcium currents (T- and L-type) exhibit distinct pattern of occurrence among “mature” taste cells, that is, cells with large \(I_{Na}\). Amiloride-sensitive sodium channels are believed to represent “sodium receptors” and to detect, at least in part, food Na\(^{+}\) (Lindemann 1996). Therefore taste cells possessing amiloride-sensitive sodium currents are likely to be

**FIG. 1.** Functional types of mature taste cells in taste buds of rat fungiform papillae. Whole cell, voltage-clamp recordings from fungiform taste cells. Cells were held at about \(-80\) mV and step depolarized (10-mV increments). Mature taste cells were endowed with large voltage-gated sodium currents \((I_{Na})\). On the basis of the amplitude of voltage-gated potassium currents (upward deflections in the records), mature taste cells could be distinguished into two subsets: “A cells,” with small potassium currents \((A)\), and “B cells,” with large potassium currents \((B)\). Current-voltage \((I-V)\) relationships for the outward (potassium) currents were obtained by measuring current amplitude \((I_m)\) at the end of 40-ms voltage steps (black dots on the current traces on the left). Points represent mean values \(\pm SE\) \((n = 33\ A\ cells; 21\ B\ cells)\). Box and whiskers plots on the right represent the amplitude distribution of potassium current \((I_K)\) evaluated at \(+50\) mV. Note that in A cells, amplitude values were all \(<2,000\ pA\) (dashed line), whereas the opposite was observed for B cells. Boxes show the middle half of the data (the 25th and 75th percentiles) and the horizontal line marks the median, whereas the “whiskers” extending from the top and the bottom of the boxes show the main body of the data. C: input resistance \((R_{input})\) and zero-current potential \((V_0)\) of A cells and B cells identified in rat fungiform taste buds. Histograms represent mean values \(\pm SE\) \((n = 25\ A\ cells; 16\ B\ cells)\). A cells differed significantly \((t\text{-test}; P < 0.001)\) from B cells for both parameters.

**FIG. 2.** Response to amiloride in taste cells of rat fungiform papillae. A: sample response to amiloride recorded from an A cell \((left)\) and from a B cell \((right)\). Cells were held at \(-80\) mV and amiloride \((1\ \mu M\ for\ A\ cell; 50\ \mu M\ for\ B\ cell)\) was bath-applied \((AM,\ boxes\ over\ records)\). Note that amiloride induced a larger reduction in the stationary inward current \((I_s,\ dashed\ line)\) in A cell but not in B cell. \(I_s\) value in A cell: about \(-195\ pA\) and about \(-20\ pA\) before and during amiloride application, respectively. \(I_s\) value in B cell: about \(-80\ pA\) and about \(-70\ pA\) before and during amiloride application, respectively. B: comparison of the amplitude of responses to 1 \(\mu M\) amiloride measured in 33 A cells and in 21 B cells. The histograms represent mean values \(\pm SE\). The difference between the amplitude values was highly significant \((t\text{-test}; P < 0.0001)\). \(I_{am}\) amplitude of the response to amiloride.
channels have been implicated in neurotransmitter release in cochlear hair cells (Brandt et al. 2003) and in retina photoreceptors (Schmitz and Witkovsky 1997). In taste cells, L-type channel has been considered as a good candidate for the influx of calcium leading to transmitter release at the synapse with sensory axons (Béhé et al. 1990; Clapp et al. 2006; DeFazio et al. 2006). Therefore taste cells endowed with L-type calcium currents are likely to be presynaptic elements. According to our findings, sodium-chemosensitive cells (A cells) and presynaptic cells (B cells) form two separate populations in rat fungiform papillae. This is not consistent with the current hypothesis of sodium transduction, which is believed to involve successive steps occurring in one single cell: influx of sodium through ASSCs, membrane depolarization, action potential firing, opening of voltage-gated calcium channels, transmitter release (reviewed by Lindemann 1996). Instead, our data support the model recently proposed by others regarding the transduction of papillae. This is not consistent with the current hypothesis of neurotransmitter release at the synapse with sensory axons (Be´he´ et al. 1990; Clapp et al. 2006; DeFazio et al. 2006). Therefore taste cells endowed with L-type calcium currents are likely to be presynaptic elements. According to our findings, sodium-chemosensitive cells (A cells) and presynaptic cells (B cells) form two separate populations in rat fungiform papillae. This is not consistent with the current hypothesis of sodium transduction, which is believed to involve successive steps occurring in one single cell: influx of sodium through ASSCs, membrane depolarization, action potential firing, opening of voltage-gated calcium channels, transmitter release (reviewed by Lindemann 1996). Instead, our data support the model recently proposed by others regarding the transduction process for sweet, bitter, and umami stimuli (Clapp et al. 2006; DeFazio et al. 2006; Huang et al. 2007) and involving separate cell groups with well-defined functions: the receptor cells, collecting information on the environmental chemical clues, and presynaptic cells, relaying that information to the nerve endings. In this model, communication between the two cell groups becomes an essential feature of taste transduction (reviewed by Roper 2007). It is therefore tempting to speculate that in rat fungiform papillae, A cells endowed with sodium receptors (ASSCs) communicate with B cells, which then could relay sensory information to nerve endings via conventional synaptic transmission.

An alternative to this model is that chemosensitive cells communicate with nerve endings through nonconventional synapses. Recent studies have shown that taste cells can release ATP [a candidate neurotransmitter in taste buds (Finger et al. 2005)] via pannexin or connexin hemichannels, without vesicular exocytosis (Huang et al. 2007; Romanov et al. 2007). Released ATP could excite directly nerve endings (Romanov et al. 2007) or affect adjacent cells connected to nerve endings through conventional synapses (Huang et al. 2007). Whether taste cells with ASSCs (A cells) are able to release ATP via hemichannels remains to be elucidated.
An interesting finding is that about half of sodium-sensitive cells expressed functional T-type calcium current (Fig. 3C). It is well known that T-type channels play an important role in the genesis of repetitive firing and pacemaking (reviewed in Perez-Reyes 2003). Repetitive firing occurs in taste cells during stimulation with Na⁺ (Avenet and Lindemann 1991; Furue and Yoshii 1997; Gilbertson et al. 1992; Varkevisser et al. 2001). Thus T-type calcium channels could be involved in defining the firing pattern of taste cells during salt transduction. In the olfactory receptor cells of the newt, studies by Kawai and co-workers (1996) have indicated that T-type channels may contribute to enhancing odor sensitivity by lowering the threshold for spike generation. In chemosensitive taste cells, T-type channels could play a similar role in modulating the sensitivity toward sodium ions.

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**REFERENCES**


