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Commentary
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Is the Amiloride-Sensitive Na^+ Channel in Taste Cells Really ENaC?

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Abstract

Among the 5 taste qualities, salt is the least understood. The receptors, their expression pattern in taste cells, and the transduction mechanisms for salt taste are still unclear. Previous studies have suggested that low concentrations of NaCl are detected by the amiloride-sensitive epithelial Na^+ channel (ENaC), which in other systems requires assembly of 3 homologous subunits (α, β, and γ) to form a functional channel. However, a new study from Lossow and colleagues, published in this issue of Chemical Senses, challenges that hypothesis by examining expression levels of the 3 ENaC subunits in individual taste cells using gene-targeted mice in combination with immunohistochemistry and in situ hybridization. Results show a lack of colocalization of ENaC subunits in taste cells as well as expression of subunits in taste cells that show no amiloride sensitivity. These new results question the molecular identity of the amiloride-sensitive Na^+ conductance in taste cells.

Key words: epithelial sodium channels, salt, taste buds

At least 2 mechanisms of salt taste detection have been identified in mammals and include amiloride-sensitive and amiloride-insensitive pathways. This dichotomy was first identified by John DeSimone and colleagues who found that chorda tympani nerve responses to NaCl were partially inhibited by the diuretic drug amiloride (Heck et al. 1984). Further experiments involving single fiber recordings from the chorda tympani nerve showed 2 distinct populations of NaCl responsive neurons. One was Na^+ selective and inhibited by low concentrations of amiloride, while the other was broadly responsive to electrolytes and insensitive to amiloride (Ninomiya and Funakoshi 1988). Amiloride is a well-known inhibitor of the epithelial Na^+ channel (ENaC), a highly Na^+-selective ion channel found in the apical membrane of most transporting epithelial cells, including kidney, lung, and colon. The channel consists of 3 homologous subunits (α, β, and γ) and all 3 subunits are required to form a functional channel (Canessa et al. 1994); αENaC is believed to be the pore-forming subunit, while the β and γ subunits are required for efficient trafficking to the apical membrane. Given the selectivity to Na^+ and the high sensitivity to amiloride, ENaC was an ideal candidate for the amiloride-sensitive Na^+ conductance in taste cells. Whole-cell patch clamp studies showed that amiloride inhibits a resting Na^+ conductance in isolated taste cells from anterior tongue in rats (Doolin and Gilbertson 1996; Bigiani and Cuoghi 2007), hamsters (Gilbertson and Fontenot 1998), and mice (Vandenbeuch et al. 2008) and immunocytochemistry (Lin et al. 1999) and reverse transcription polymerase chain reaction (RT-PCR) (Kretz et al. 1999; Shigemura et al. 2005) showed expression of all 3 subunits in tongue epithelium. Taste nerve recordings show that the amiloride-sensitive conductance is limited to anterior tongue, although RT-PCR showed expression of αENaC in all taste fields. Yoshida et al. (2009) combined electrophysiology with single cell RT-PCR in fungiform taste buds and found that about half of the amiloride-sensitive cells expressed αENaC but few expressed other subunits. Although ENaC was generally assumed to be the basis of amiloride-sensitive NaCl taste, confirmation of function requires knockout of the channel. In 2010, Charles Zuker and colleagues genetically engineered mice lacking αENaC in taste cells, which resulted in complete abolishment of amiloride-sensitive salt taste and a loss of behavioral attraction for low concentrations of NaCl (Chandrashekar et al. 2010). These results confirmed that at

least αENaC is required for amiloride-sensitive salt taste and suggested it was the basis of appetitive salt taste.

A new study published in this issue of Chemical Senses calls into serious question the role of ENaC in amiloride-sensitive salt taste. Lossow et al. (2020) examined the expression pattern of individual ENaC subunits in the different taste cell types. Taste cells consist of 3 cell types that are distinguished by morphological, molecular, and functional characteristics (Roper and Chaudhari 2017). Type I cells are generally believed to have a support or glial-like function, while Type II cells transduce bitter, sweet, or umami stimuli via G-protein-coupled taste receptors. Type III cells respond to sour stimuli and a subset transduce amiloride-insensitive salt taste (Lewandowski et al. 2016; Larson et al. 2020). To examine expression of the individual subunits in taste cells, Lossow et al. (2020) engineered mice to express fluorescent reporter proteins in cells expressing αENaC or βENaC; αENaC cells expressed GFP while βENaC cells expressed tdRFP. γENaC was identified with immunocytochemistry and riboprobes. These constructs were validated in kidney tissue, which verified that all 3 ENaC subunits were coexpressed in each of the transporting epithelial cells. Hundreds of cells were studied in sections of taste tissue from fungiform, circumvallate, foliate, and soft palate. αENaC, identified by green fluorescence, was found primarily in Type III taste cells. βENaC, identified by red fluorescence, was found primarily in Type I and a few Type II cells. γENaC was found primarily in Type II cells, with small amounts in Type III and Type I cells. Interestingly, not a single taste cell expressed both the green (αENaC) and red (βENaC) reporters, questioning the stoichiometry of the functional channel in taste cells. Further, αENaC, thought to be required for amiloride sensitivity, was expressed more abundantly in circumvallate taste buds than fungiform taste buds, although nerve recordings and patch clamp studies suggest circumvallate taste buds have no amiloride sensitivity. Previous studies have shown that amiloride-sensitive NaCl responses occur in a cell type that is neither Type II nor Type III cells (Chandrashekar et al. 2010; Roebber et al. 2019), but possibly Type I cells (Vandenbeuch et al. 2008) or a unique cell type.

If ENaC subunits do not colocalize in taste cells, what is the molecular identity of the amiloride-sensitive channel in taste cells? Clearly αENaC is required since the amiloride-sensitive responses are abolished in the knockout. One possibility, offered by the authors, is Type I cells may express low levels of αENaC and γENaC that were not detected by their reporter constructs or immunohistochemistry. These would then combine with βENaC and form a functional channel in Type I cells. Alternatively, a different ENaC subunit might be involved in the taste channel. Human taste buds express δENaC instead of αENaC so other amiloride-sensitive subunit compositions are possible (Giraldez et al. 2012; Ji et al. 2012). Amiloride, however, has little effect on salt taste in humans (Desor and Finn 1989; Halpern et al. 1995). Of note, amiloride sensitivity varies with the background of mice. The mice in the present study were produced in the 129 line and crossed into the C57Bl6 line. Mice of the 129 background are known to have lower amiloride sensitivity (Shigemura et al. 2008) and thus there may be fewer cells that would be expected to be amiloride sensitive. Clearly further studies are needed to resolve these apparent discrepancies. These new reporter mice should be very useful tools for both physiological studies and single cell transcriptome profiling, possibly revealing the identity of new ENaC subunits.

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