Published in final edited form as:

Pflugers Arch. 2021 January ; 473(1): 3–13. doi:10.1007/s00424-020-02464-4.

Taste transduction and channel synapses in taste buds

Akiyuki Taruno[1,2], **Kengo Nomura**[1], **Tsukasa Kusakizako**[3], **Zhongming Ma**[4], **Osamu Nureki**[3], **J. Kevin Foskett**[4,5]

[1]Department of Molecular Cell Physiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan

[2]Japan Science and Technology Agency, PRESTO, Kawaguchi, Saitama, Japan

[3]Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan

[4]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

[5]Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Abstract

The variety of taste sensations, including sweet, umami, bitter, sour, and salty, arises from diverse taste cells, each of which expresses specific taste sensor molecules and associated components for downstream signal transduction cascades. Recent years have witnessed major advances in our understanding of the molecular mechanisms underlying transduction of basic tastes in taste buds, including the identification of the bona fide sour sensor H^+ channel OTOP1, and elucidation of transduction of the amiloride-sensitive component of salty taste (the taste of sodium) and the TAS1R-independent component of sweet taste (the taste of sugar). Studies have also discovered an unconventional chemical synapse termed “channel synapse” which employs an action potential-activated CALHM1/3 ion channel instead of exocytosis of synaptic vesicles as the conduit for neurotransmitter release that links taste cells to afferent neurons. New images of the channel synapse and determinations of the structures of CALHM channels have provided structural and functional insights into this unique synapse. In this review, we discuss the current view of taste transduction and neurotransmission with emphasis on recent advances in the field.

Keywords

Taste; Sensory; Synapse; Ion channel; CALHM

✉Akiyuki Taruno, taruno@koto.kpu-m.ac.jp.

Conflict of interest The authors declare that they have no conflict of interest.

Introduction

Taste is a chemosensation perceived on the tongue and provoked by chemical compounds contained in food and drinks. A chemical that produces a taste sensation is called a tastant. A vast variety of tastants can be categorized into five groups depending on the quality of the evoked taste sensation: sweet, umami, bitter, salty, and sour. Animals are attracted to sweet and umami tastes produced by caloric nutrients, sugars, and amino acids, respectively. Bitter and sour tastes are indicative of potentially toxic and spoiled ingredients, respectively, and cause innate avoidance. The salty taste of NaCl can be attractive or aversive depending on its concentration in order to fine-tune the body electrolyte homeostasis. Thus, taste plays pivotal roles in survival by controlling dietary choices. Because the epidemic of overnutrition, which is closely associated with diet, is a global health problem in modern societies, understanding taste is of considerable importance.

The taste sensor organ, the taste bud, is a cluster of elongated cells mainly found in the lingual epithelium. Each of the five basic tastes is sensed by dedicated taste cells that are morphologically and functionally categorized into three types: I, II, and III (Fig. 1a). Type II cells are further categorized into sweet, umami, and bitter cells, depending on the specific taste receptor molecules expressed in the apical membrane. A remarkable feature of type II cells is that, instead of exocytosis of synaptic vesicles, they communicate with the afferent neurons by an unconventional chemical synapse termed a “channel synapse” that involves an action potential-activated calcium homeostasis modulator 1/3 (CALHM1/3) ion channel as the neurotransmitter release machinery [22, 38, 48, 72, 91]. Type III cells respond to sour taste using OTOP1 as the primary sour sensor ion channel [98], and they form conventional vesicular synapses with afferent nerves. Type I cells function like glial cells that extend thin processes that wrap around type II and III cells and thereby electrically and chemically isolate them. Of salty taste, the amiloride-sensitive component is mediated by as-yet unclassified taste cells with channel synapses [60], while amiloride-insensitive high-salt taste relies on bitter and sour cells [62]. Numerous studies over the last decades have identified many key molecules responsible for taste reception in taste buds, but our understanding of taste mechanisms remains incomplete and is still rapidly being updated. In this review, we summarize the current understanding of signal transduction and transmission of five basic tastes in taste buds, with emphasis on the recent advances in the field.

Sour taste

Extracellular and intracellular protons are the primary stimulant for sour-sensing type III cells. Therefore, H^+-releasing chemicals (i.e., acids) serve as sour tastants. Despite speculation that ionic taste stimuli are transduced by ion channels, the molecular identity of the sour sensor channel remained elusive until recently. Although many cation channels were proposed as candidates, including ASICs [99], HCNs [83], and TRP channels PKD2L1, and PKD1L3 [34, 36], subsequent studies failed to support their involvement in sour taste [33, 68, 69]. Nevertheless, the complete loss of gustatory nerve responses to acids by the ablation of $PKD2L1^+$ cells identified PKD2L1 as a marker gene for sour-tasting type III cells [34]. In the last decade, a series of studies by Emily Liman and colleagues have taken advantage of this molecular identification to reveal an elaborate

sour transduction cascade that involves a previously unrecognized H^+-selective ion channel, otopetrin1 (OTOP1) as the bona fide sour sensor channel (Fig. 1b). The initial event in sour transduction is a H^+ current in the apical membrane through a unique Zn^{2+}-sensitive H^+-selective conductance that can depolarize the cells sufficiently to drive action potential generation [8, 13]. Comparative transcriptome analysis between $PKD2L1^+$ and $TRPM5^+$ taste cells identified OTOP1 as a novel H^+-selective ion channel protein required for the H^+ current specifically in type III cells [98]. The structure of zebrafish OTOP1 resolved by single-particle cryo-electron microscopy (EM) provided insights into how OTOP1 channels selectively conduct protons [76]. *Otop1* knockout (KO) mice had severely attenuated type III cell and gustatory neuron responses to acids, confirming OTOP1 as the sour sensor [92, 114]. The H^+ influx through OTOP1 results in depolarization and a decrease in intracellular pH (pH^i), leading to inhibition of a pH_i-sensitive resting K^+ conductance mediated by $K_{ir2.1}$ channels that enhances depolarization [110]. Type II cells also have $K_{ir2.1}$ currents that account for their responsiveness to intracellular acidification [110]. Whereas they are larger than those in type III cells, the higher input resistance of type III cells results in a stronger depolarization. Together, OTOP1 and $K_{ir2.1}$ channels work in concert to transduce acid stimuli into a membrane depolarization, triggering action potentials that activate voltage-gated Ca^{2+} channels for Ca^{2+}-dependent exocytotic release of neurotransmitters at synapses with gustatory neurons. Although adenosine triphosphate (ATP) and 5-HT have been implicated [28, 41], the primary neurotransmitter linking type III cells and afferent neurons remain to be established. Weak acids such as acetic acid are perceived to be more sour than strong acids at the same pH, likely because protonated forms of weak acids can permeate the plasma membrane and release H^+ in the cytoplasm, leading to additional intracellular acidification.

Notably, type III cells respond not only to acids but also to carbonation [12], high concentrations of salts [62], and even water [118]. Furthermore, acids stimulate both type III taste cells and trigeminal nociceptors in the oral cavity [95, 105]. Optogenetic activation of type III cells evoked aversive and attractive behaviors in mice, possibly depending on the physiological conditions [106, 118]. Thus, it remains to be resolved how neural outputs from type III cells contribute to our perception of these taste qualities.

Sweet, umami, and bitter tastes

Sweet, umami, and bitter tastants are sensed by specific G protein-coupled receptors expressed in dedicated type II cells: sweet, umami, and bitter cells. The TAS1R family members constitute the sweet and umami receptors [57, 58, 117]. Sweet cells use a heterodimer of TAS1R2 and TAS1R3 (TAS1R2/3) for detecting natural and artificial sweeteners, whereas umami cells employ a heterodimer of TAS1R1 and TAS1R3 (TAS1R1/3) for detecting various L-amino acids and nucleotides. Meanwhile, each of the bitter cells in humans and mice expresses ~ 25 and ~ 35 TAS2Rs, respectively to detect numerous, chemically diverse bitter tastants [1, 11]. Expression of sweet, umami, and bitter receptors in taste buds is segregated to distinct cells, although identification of cells expressing all TAS1R subunits has been reported [40]. Downstream of the taste receptors, sweet, umami, and bitter cells share a similar intracellular signaling cascade to transduce chemical stimuli into action potential firing. Binding of tastants to the taste

receptors initiates the following signaling cascade (Fig. 1c): (i) activation of phospholipase Cβ2 (PLCβ2) [115], (ii) production of inositol 1,4,5-trisphosphate (IP_3), (iii) an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) due to Ca^{2+} release from the endoplasmic reticulum through type 3 IP_3 receptor Ca^{2+} channels (IP_3R3) [31], (iv) $Ca^{2+}{}_i$-dependent activation [32, 43, 66, 116] of plasma membrane monovalent cation-selective ion channels TRPM5 [65, 115] to generate a graded depolarization, and (v) Na^+ action potential generation [113]. Of note, previous studies involving *Trpm5* KO mice reported a TRPM5-independent $Ca^{2+}{}_i$-activated ion channel in type II cells with a much lower affinity for $Ca^{2+}{}_i$ than TRPM5 [116] and residual gustatory nerve and behavioral responses to sweet, umami, and bitter [18]. A recent study provided evidence that TRPM4, another Ca^{2+}-activated TRP channel, mediated the TRPM5-independent responses [26]. These electrically excited type II cells transmit taste information to the afferent neurons via a channel synapse, as described below.

In addition to the canonical TAS1R-mediated mechanisms, some studies have shown that there are TAS1R-independent receptor mechanisms for sweet and umami tastants. Indeed, *Tas1r3* KO mice lost the perception of artificial sweeteners and maintained reduced but significant responses to sugars, especially glucose, and to glutamate [19]. The TAS1R3-independent detection of glucose and glutamate involves sodium-glucose cotransporter 1 (SGLT1) [109, 111] and metabotropic glutamate receptors (mGluR1 and mGluR4) [14, 108], respectively. It is believed that SGLT1-mediated glucose-coupled Na^+ influx provides a supra-threshold depolarization for action potential generation, based on the observation that the non-metabolizable glucose analog, methyl-a-D-glucopyranoside, mimicked the action of glucose [109]. GLUT family glucose transporters and ATP-sensitive K^+ channels (K_{ATP}) may also contribute to depolarization by constituting a metabolic pathway, in which monosaccharides transported into cells by SGLT1 and GLUTs are metabolized to raise the ratio of ATP to ADP, leading to the closure of K_{ATP} channels and thereby cause membrane depolarization [111]. Since SGLT1, GLUT4, and K_{ATP} channels are preferentially expressed in $TAS1R3^+$ taste cells [111], the SGLT1-dependent sugar detection mechanism involving glucose-coupled Na^+ influx may explain why a pinch of salt (NaCl) enhances sweetness in food. Curiously, intestinal SGLT1 was recently identified as a glucose sensor that initiates a gut-to-brain post-ingestive sugar sensing pathway critical for the development of sugar preference [86]. Thus, SGLT1 is a key molecule for detecting sugars in both the mouth and gut.

Salty taste

Sodium is an essential mineral because it is the major cation of the extracellular fluid and thereby maintains the body fluid volume homeostasis and various cellular functions. On the other hand, a high dietary intake of sodium is harmful because it is associated with elevated blood pressure, a risk factor for cardiovascular diseases [54, 84]. To control the amount of sodium ingested within a proper range, many mammals are equipped with two salt taste mechanisms: sodium taste [10] and high-salt taste [62].

Sodium taste

The remarkable features of sodium taste are the strict selectivity to sodium salts and sensitivity to a diuretic, amiloride, and it typically evokes behavioral attraction. The pioneering work of DeSimone and colleagues [30] and subsequent contributions from many others [75] culminated in the identification of the sensor for sodium taste [10] as the amiloride-sensitive epithelial Na^+ channel (ENaC), which is composed of α, β, and γ subunits [9, 61] and localizes in apical membranes of taste cells [112]. However, which taste cells are involved, and the details of the intracellular signaling mechanisms underlying sodium taste have remained long-standing enigmas. Sodium taste cells are present in taste buds in fungiform papillae of the anterior tongue but not in circumvallate papillae of the posterior tongue [39, 59]. Until recently, it had been believed that sodium taste cells are a subset of type I cells [15, 37, 56, 79] due to the absence of co-expression of all three ENaC subunits in taste cell populations expressing marker proteins of type II and III cells (TRPM5 and Car4, respectively) [10]. However, the properties of type I cells are apparently not compatible with taste sensor functions, as they are reportedly electrically non-excitable [51]. How are sodium stimuli transduced in sodium taste cells? One study detected an amiloride-sensitive current ($I_{amiloride}$), an index of ENaC activity, only in taste cells lacking voltage-gated Na^+ currents (I_{Nav}) [102], while others reported cells having both $I_{amiloride}$ and I_{Nav} [23, 52]. Thus, it has been unclear whether sodium taste cells are electrically excitable. Furthermore, the involvement of Ca^{2+} signals in sodium taste transduction has been controversial [6, 10], and the neurotransmission mechanisms from sodium taste cells to afferent neurons remained unexplored. A recent report [60] identified the cells responsible for sodium taste and elucidated the mechanisms of sodium taste transduction (Fig. 1c). Patch-clamp electrophysiology and Ca^{2+} imaging in ENaCα^+ cells from fungiform taste buds of mice expressing GCaMP3 under the ENaCα promoter found $I_{amiloride}$ in two functionally distinct cell types: electrically excitable and non-excitable ones. In the excitable cells, Na^+ influx through ENaCs evoked action potentials without changes in $[Ca^{2+}]_i$. Whole-cell current-clamp recordings with strong intracellular Ca^{2+} chelation further demonstrated that $I_{amiloride}$ alone was sufficient to evoke action potentials without the aid of Ca^{2+} signals. Thus, ENaC-mediated Na^+ influx alone drives action potential generation. The excitable, but not the non-excitable cells with $I_{amiloride}$, expresses the CALHM1/3 ion channel, the neurotransmitter-release channel found in type II cells [48]. Importantly, conditional KO of ENaCα in CALHM1$^+$ cells abolished amiloride-sensitive gustatory nerve responses and attenuated behavioral attraction to NaCl, indicating that the excitable cells with $I_{amiloride}$ mediate appetitive sodium taste. Consistent with earlier studies suggesting a role of CALHM channels in sodium taste [4, 97], global *Calhm3* KO mice lost perception of sodium taste, and CALHM1 was localized at points of contact between sodium taste cells, identified as ENaCα^+ CALHM1$^+$ cells, and P2X2$^+$ afferent nerves (Fig. 2a), a structural feature of the CALHM1/3 channel synapse in type II cells, as described below. These findings suggested that sodium taste cells employ a channel synapse for neurotransmission to afferent neurons. Together, ENaCα^+ CALHM1/3$^+$ cells constitute sodium taste cells, and ENaC-mediated entry of oral Na^+ elicits a supra-threshold depolarization for action potentials driving voltage-dependent neurotransmitter release via the CALHM1/3 channel synapse. All steps in sodium transduction are voltage driven and independent of Ca^{2+} signals, in contrast to other types of taste cells. Of note, although the requirement of ENaCα

was established by the loss of sodium taste in ENaCα KO animals [10, 60], a recent study failed to find taste cells expressing all three ENaC subunits (α, β, and γ), questioning the stoichiometry of the functional amiloride-sensitive Na^+ channel in sodium taste cells [44].

Are sodium taste cells, which are distinct from type II and III cells [10], a subset of type I cells? Sodium cells identified as ENaCα$^+$ CALHM3$^+$ cells do not express NTPDase2, a type I cell marker [60]. Thus, sodium taste cells appear to define a previously unidentified taste cell population in addition to the widely known type I/II/III taste cell classes, suggesting a greater diversity than previously realized in fungiform taste cells. The taste cell classification has mainly been developed in circumvallate taste buds, and it is unclear whether it applies to fungiform taste buds. Further studies are needed to clarify the diversity in fungiform taste cells and how sodium taste cells are classified among them.

Importantly, while the role of ENaC in rodent salty taste has been established, amiloride-sensitivity of perceived saltiness in humans has been controversial [2, 29, 50, 63, 64, 77, 81, 93, 94]. This may reflect an unconventional subunit composition or subcellular localization of ENaC in human taste cells [82, 104]. Direct evidence supporting ENaC as the principal sensor of human salty taste has yet to be demonstrated [5].

High-salt taste

Alternatively, perceived saltiness is determined by multiple salt taste pathways that are subject to dynamic modulation by experience or the internal state. Indeed, in addition to sodium taste, there is another taste pathway responding to salts called high-salt taste. High-salt taste has a higher threshold concentration than that of sodium taste and is evoked by various salts including NaCl and KCl. This pathway is also known as amiloride-insensitive salty taste because it is not affected by amiloride. In taste buds, the perception of high salt relies on bitter-sensing type II cells and sour-sensing type III cells and typically evokes behavioral avoidance [62]. Recruiting multiple cell types likely involves multiple receptor mechanisms. One or more bitter receptors and carbonic anhydrase 4 were initially suggested in bitter and sour cells, respectively [62]. A more recent study [70] suggested Cl^- as a key determinant of high-salt responses in type II cells but ruled out the involvement of Cl^- channels and transporters (see also [27]). It has also been suggested that a subset of type III cells responds to high-salt stimuli through multiple mechanisms, including osmotically activated Na^+-conducting ion channels [42]. Due to these seemingly contradictory findings, the nature of high-salt receptors remains elusive. Adding another layer of complexity, CALHM1/3-dependent, ENaC-independent salt attraction behavior with a high threshold concentration was recently observed in salt-depleted mice [60]. It is unknown whether this attractive high-salt response represents a novel salty taste mechanism or is a result of the central modulation of preference for a known high-salt taste pathway. Identification of high-salt sensors and understanding of modulation and integration of each salt taste pathway at the periphery and in the brain are imperative in order to understand the human salty taste and devise strategies to reduce salt consumption.

Channel synapse

Type II cells transmit taste information to the gustatory neurons by release of neurotransmitter, but the mechanism underlying this neurotransmission was a long-standing mystery. The most widespread mechanism of chemical neurotransmission is that chemicals contained in synaptic vesicles (a.k.a. neurotransmitters) are released into the synaptic cleft by Ca^{2+}-activated exocytosis and bind to specific receptor proteins expressed in the plasma membrane of a postsynaptic cell. However, type II cells do not possess essential components of conventional synapses such as synaptic vesicles and SNAP25, a key protein for Ca^{2+}-activated transmitter exocytosis [21]. How do taste cells lacking synaptic vesicles mediate neurotransmission?

Discovery of the neurotransmitter-release channel, CALHM1/3

ATP was suggested to be the primary neurotransmitter linking taste cells to sensory nerve fibers. Type II cells release ATP in response to taste stimuli [28, 35, 55, 73, 91], afferent gustatory neurons innervating taste cells express ATP receptors composed of P2X2/P2X3 [7, 49], and double KO of *P2X2* and *P2X3* abolished taste-evoked responses to sweet, bitter, and umami substances [28]. NTPDase2 is expressed in type I cells and likely contributes to taste perception by degrading extracellular ATP to ADP [3, 101]. Thus, ATP meets most criteria for a neurotransmitter, including release, presence of specific receptors, and mechanisms for clearance [28]. The role of ATP in the sour transmission is unclear. Although *P2X2/P2X3* double KO mice lost sour perception [28], ATP release has not yet been detected from type III cells. However, the mechanism for type III cell neurotransmitter release has been well understood. Electrophysiological estimates of the changes in membrane surface area from fluctuations of whole-cell membrane electrical capacitance provided functional evidence that type III cells responded to membrane depolarization by increasing the cell surface area due to the insertion of synaptic vesicles in regulated exocytosis [103]. These functional data are in agreement with anatomical evidence that a conventional vesicular synapse exists in type III cells [17]. However, these functional and anatomical features were not detected in type II cells. The implication was that type II cells use non-exocytotic mechanisms to release ATP, possibly involving ion channels. There are five known classes of ATP-permeable ion channels, including connexin hemichannels, pannexin 1, maxi-anion channels, volume-regulated anion channels, and CALHM channels (reviewed in [88]). Although pannexin 1 was initially proposed as the ATP-release channel in type II cells [20, 35], genetic KO studies [71, 96, 100] precluded its involvement since ATP release from type II cells and taste perception were both intact in *Panx1* KO mice. Connexins were also implicated in the type II cell ATP release [73, 74], but their contributions to taste perception have not been tested using KO models. Meanwhile, CALHM1 was identified as an essential component of the ATP release machinery in type II cells [91].

CALHM1 was originally identified as an essential component of a previously uncharacterized ion channel that controls susceptibility to Alzheimer's disease [24]. Subsequently, it was established as a pore-forming subunit of a plasma membrane non-selective voltage-gated ion channel with a wide pore [47, 80]. Remarkably, activation of CALHM1 leads to translocation of ATP from the cytoplasm to the extracellular milieu,

identifying CALHM1 as a novel voltage-gated ATP-release channel [91]. In taste buds, CALHM1 expression was found selectively in type II cells [53], and *Calhm1* KO abolished non-selective voltage-gated currents and taste-evoked ATP release in type II cells and severely impaired gustatory nerve and behavioral responses to sweet, bitter, and umami tastes. These findings established CALHM1 as an essential component of the type II cell ATP-release channel [91]. However, the kinetics of voltage-dependent activation of CALHM1 expressed in heterologous systems ($\tau > 0.5$ s) are too slow to be activated by rapid Na^+ action potentials that trigger ATP release in taste cells [55, 91]. Furthermore, the endogenous taste cell ATP-release channel exhibits considerably faster activation kinetics ($\tau \sim 10$ ms) than those of recombinant CALHM1 [46, 74]. In addition, type II cell ATP release, but not heterologously expressed CALHM1 ion currents, is inhibited by carbenoxolone [20, 35, 47, 55]. Together these resulted suggested that whereas CALHM1 was essential for type II cell-mediated ATP release, other components were likely to also play a role.
A recent study [48] discovered that a CALHM1 homolog, CALHM3, hetero-oligomerizes with CALHM1 to form a novel voltage-gated ATP-permeable CALHM1/3 channel with fast activation kinetics ($\tau \sim 10$ ms) and sensitivity to carbenoxolone, which match the properties of the type II cell ATP-release channel. Of note, CALHM1/3 has fast gating kinetics comparable with typical voltage-gated K^+ channels and is currently the only known ATP-release channel that can potentially be activated by action potentials. In mice, CALHM1 and CALHM3 were co-expressed in type II cells, and *Calhm3* KO abolished the endogenous type II cell ATP channel current and taste-evoked ATP release and markedly impaired gustatory nerve and behavioral responses to sweet, umami, and bitter tastes. Thus, a CALHM1/3 channel was established as the bona fide ATP-release channel required for rapid neurotransmission in type II cells (Fig. 1c). More recently, CALHM1/3 was also discovered to mediate neurotransmission of sodium taste [60] (Fig. 1c). This identification of an action potential-activated neurotransmitter-release channel establishes the molecular mechanism of how taste cells lacking synaptic vesicles release neurotransmitters. This represents a new mode of chemical synapse unique to taste cells, which we term “channel synapse” in contrast to the conventional “vesicular synapse” (Fig. 1).

Structure of the channel synapse

Recently, super-resolution immunohistochemical analyses identified the localization of CALHM1 in the basolateral membrane of both type II cells and sodium taste cells in discrete puncta localized close to P2X2-expressing afferent nerve fibers [38, 60, 72]. Furthermore, ultrastructural immunohistochemistry revealed that large “atypical” mitochondria are consistently juxtaposed closely to membrane areas accumulating CALHM1 [72]. Pharmacological inhibition of ATP production in mitochondria blocked taste cell ATP release, suggesting that the immediate source of released ATP was the atypical mitochondrion rather than a cytoplasmic pool [72]. The close apposition of the ATP source, ATP-release channel, and ATP receptors defines the structure of the channel synapse, and is thought to enable stable, spatially localized neurotransmitter release in the absence of synaptic vesicles (Fig. 2). This spatial arrangement of the three components is unlikely to be a product of chance and likely involves scaffold proteins, as suggested by the uniform distances between the mitochondrial outer membrane and plasma membrane (20~40 nm)

and between a taste cell and a nerve (10~15 nm) [72]. Identification of these scaffold proteins will facilitate understanding of the channel synapse formation.

Structure of CALHM channels

The CALHM channel family comprises six members, CALHM1-6 [24], among which CALHM1, CALHM2, and CALHM3 have been implicated in physiological and pathological processes, including taste neurotransmission [48, 60, 89, 91], neuronal excitability [47], Alzheimer's disease [24], and depression [45]. Recent studies by single-particle cryo-EM have revealed structures of several CALHM channels and provided insights into how CALHMs function as ion channels [16, 22, 25, 67, 85, 107]. As previously predicted [24, 47, 80, 87, 90], protomers of all CALHM channels harbor four transmembrane (TM) helices and cytoplasmic C-terminal helices (CTH). This membrane topology is reminiscent of other large pore-forming channel proteins such as connexins, innexins, pannexin1, and LRRC8. However, viewed from the extracellular side, the anticlockwise arrangement of TM1, TM2, TM3, and TM4 helices in CALHMs distinguishes them from other large-pore channels, all of which have a clockwise arrangement. CALHMs exhibit various oligomeric assemblies from octamers to dodecamers and consequently different sizes, although the physiological relevance of the diverse oligomeric assemblies is not well understood [16, 22, 25, 67, 85, 107]. Unlike the hexameric assembly previously suggested for human CALHM1 and CALHM1/3, three studies revealed octamer assembly of killifish CALHM1 [22] (Fig. 3a), chicken CALHM1 [85], and zebrafish CALHM1 [67]. These studies suggest that human CALHM1 is also likely an octamer since the residues responsible for subunit interactions are highly conserved in the CALHM1 orthologs [22, 85]. Furthermore, based on the sequence similarity between CALHM1 and CALHM3, the CALHM1/3 channel is speculated to be a hetero-octamer [22]. The pore architecture has been resolved in the highest detail in killifish CALHM1, where the N-terminal helix (NTH) preceding TM1 was successfully visualized [22]. Killifish CALHM1 has a channel pore along a central axis perpendicular to the membrane, and the NTH forms a single constriction site in the pore with the narrowest diameter of 15.7 Å (Fig. 3b). NTH-truncated mutant channels showed enhanced ATP-release activities, supporting the role of the NTH in channel pore architecture [22]. The observed diameter is close to the value functionally estimated for human CALHM1 (~ 14 Å) [80] and is larger than the size of an ATP molecule (~ 12 Å). Human CALHM1 allows permeation of both cations and anions with relative permeabilities of $P_{Ca}:P_{Na}:P_{K}:P_{Cl}$ = 11:1:1.17:0.56 [47]. The pore-facing residues in the NTH are neutral, which may account for the weak charge selectivity. Thus, the observed pore architecture of killifish CALHM1 is consistent with the functional pore properties of human CALHM1. These results provide important insights into the structure and ATP permeation of the neurotransmitter-release channel in taste cells, CALHM1/3. Nevertheless, the structure of the heteromeric CALHM1/3 awaits resolution. Also, CALHM1 and CALHM3 do not possess canonical voltage-sensor domains, but CALHM1/3 is rapidly activated by depolarization [48]. Further studies are required to identify the structural basis of the voltage-dependent gating of CALHM channels. Finally, although a putative closed-state structure of CALHM2 has been reported [16], the mechanisms involved in closing CALHM1 and CALHM1/3 also await resolution.

Conclusion

The last several years have seen important advances in our understanding of the transduction mechanisms employed in the perception of basic tastes. Although we now have a fairly good molecular-level understanding of how taste cells transduce sweet, umami, bitter, sour, and sodium tastes, the identification of high-salt taste receptors and transduction mechanisms is pivotal to comprehend and control human salty taste. The discovery of the channel synapse provides a new paradigm for the mechanisms of neuronal chemical communication. In addition to sweet, umami, bitter, and sodium taste, a recent study implicated the channel synapse in the neurotransmission of fat taste, a less studied taste quality [78]. Thus, the channel synapse may mediate all taste qualities excluding sour. How does the channel synapse benefit gustation? Where else does it function outside the tongue? These questions remain to be answered.

Acknowledgments

We thank Emily R. Liman (USC) for the critical reading of the manuscript.

Funding

This work was supported by JST PRESTO JPMJPR1886 (A.T.); JSPS KAKENHI 16H06294 (O.N.); 19H03819 and 20K04908 (A.T.); Salt Science Research Foundation 18C2, 19C2, and 20C2 (A.T.); NIH R01DC018278 (Z. M., J. K. F.); and Platform Project for Supporting Drug Discovery and Life Science Research [Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS)] from AMED, under grant number JP19am01011115 (support number 1111).

References

1. Adler E, Hoon MA, Mueller KL, Chandrashekar J, Ryba NJ, Zuker CS (2000) A novel family of mammalian taste receptors. Cell 100:693–702 [PubMed: 10761934]
2. Anand KK, Zuniga JR (1997) Effect of amiloride on suprathreshold NaCl, LiCl, and KCl salt taste in humans. Physiol Behav 62:925–929 [PubMed: 9284519]
3. Bartel DL, Sullivan SL, Lavoie EG, Sevigny J, Finger TE (2006) Nucleoside triphosphate diphosphohydrolase-2 is the ecto-ATPase of type I cells in taste buds. J Comp Neurol 497:1–12 [PubMed: 16680780]
4. Bigiani A (2017) Calcium homeostasis modulator 1-like currents in rat fungiform taste cells expressing amiloride-sensitive sodium currents. Chem Senses 42:343–359 [PubMed: 28334404]
5. Bigiani A (2020) Does ENaC work as sodium taste receptor in humans? Nutrients 12:1195
6. Bigiani A, Cuoghi V (2007) Localization of amiloride-sensitive sodium current and voltage-gated calcium currents in rat fungiform taste cells. J Neurophysiol 98:2483–2487 [PubMed: 17686911]
7. Bo X, Alavi A, Xiang Z, Oglesby I, Ford A, Burnstock G (1999) Localization of ATP-gated P2X2 and P2X3 receptor immunoreactive nerves in rat taste buds. Neuroreport 10:1107–1111 [PubMed: 10321492]
8. Bushman JD, Ye W, Liman ER (2015) A proton current associated with sour taste: distribution and functional properties. FASEB J 29:3014–3026 [PubMed: 25857556]
9. Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, Rossier BC (1994) Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits. Nature 367:463–467 [PubMed: 8107805]
10. Chandrashekar J, Kuhn C, Oka Y, Yarmolinsky DA, Hummler E, Ryba NJ, Zuker CS (2010) The cells and peripheral representation of sodium taste in mice. Nature 464:297–301 [PubMed: 20107438]
11. Chandrashekar J, Mueller KL, Hoon MA, Adler E, Feng L, Guo W, Zuker CS, Ryba NJ (2000) T2Rs function as bitter taste receptors. Cell 100:703–711 [PubMed: 10761935]

12. Chandrashekar J, Yarmolinsky D, von Buchholtz L, Oka Y, Sly W, Ryba NJ, Zuker CS (2009) The taste of carbonation. Science 326:443–445 [PubMed: 19833970]
13. Chang RB, Waters H, Liman ER (2010) A proton current drives action potentials in genetically identified sour taste cells. Proc Natl Acad Sci U S A 107:22320–22325 [PubMed: 21098668]
14. Chaudhari N, Landin AM, Roper SD (2000) A metabotropic glutamate receptor variant functions as a taste receptor. Nat Neurosci 3:113–119 [PubMed: 10649565]
15. Chaudhari N, Roper SD (2010) The cell biology of taste. J Cell Biol 190:285–296 [PubMed: 20696704]
16. Choi W, Clemente N, Sun W, Du J, Lu W (2019) The structures and gating mechanism of human calcium homeostasis modulator 2. Nature 576:163–167 [PubMed: 31776515]
17. Clapp TR, Yang R, Stoick CL, Kinnamon SC, Kinnamon JC (2004) Morphologic characterization of rat taste receptor cells that express components of the phospholipase C signaling pathway. J Comp Neurol 468:311–321 [PubMed: 14681927]
18. Damak S, Rong M, Yasumatsu K, Kokrashvili Z, Perez CA, Shigemura N, Yoshida R, Mosinger B Jr, Glendinning JI, Ninomiya Y, Margolskee RF (2006) Trpm5 null mice respond to bitter, sweet, and unrami compounds. Chem Senses 31:253–264 [PubMed: 16436689]
19. Damak S, Rong M, Yasumatsu K, Kokrashvili Z, Varadarajan V, Zou S, Jiang P, Ninomiya Y, Margolskee RF (2003) Detection of sweet and umami taste in the absence of taste receptor T1r3. Science 301:850–853 [PubMed: 12869700]
20. Dando R, Roper SD (2009) Cell-to-cell communication in intact taste buds through ATP signalling from pannexin 1 gap junction hemichannels. J Physiol 587:5899–5906 [PubMed: 19884319]
21. DeFazio RA, Dvoryanchikov G, Maruyama Y, Kim JW, Pereira E, Roper SD, Chaudhari N (2006) Separate populations of receptor cells and presynaptic cells in mouse taste buds. J Neurosci 26:3971–3980 [PubMed: 16611813]
22. Demura K, Kusakizako T, Shihoya W, Hiraizumi M, Nomura K, Shimada H, Yamashita K, Nishizawa T, Taruno A, Nureki O (2020) Cryo-EM structures of calcium homeostasis modulator channels in diverse oligomeric assemblies. Sci Adv 6:eaba8105 [PubMed: 32832629]
23. Doolin RE, Gilbertson TA (1996) Distribution and characterization of functional amiloride-sensitive sodium channels in rat tongue. J Gen Physiol 107:545–554 [PubMed: 8722566]
24. Dreses-Werringloer U, Lambert JC, Vingtdeux V, Zhao H, Vais H, Siebert A, Jain A, Koppel J, Rovelet-Lecrux A, Hannequin D, Pasquier F, Galimberti D, Scarpini E, Mann D, Lendon C, Campion D, Amouyel P, Davies P, Foskett JK, Campagne F, Marambaud P (2008) A polymorphism in CALHM1 influences Ca^{2+} homeostasis, Abeta levels, and Alzheimer's disease risk. Cell 133:1149–1161 [PubMed: 18585350]
25. Drozdzyk K, Sawicka M, Bahamonde-Santos MI, Jonas Z, Deneka D, Albrecht C, Dutzler R (2020) Cryo-EM structures and functional properties of CALHM channels of the human placenta. Elife 9:e55853 [PubMed: 32374262]
26. Dutta Banik D, Martin LE, Freichel M, Torregrossa AM, Medler KF (2018) TRPM4 and TRPM5 are both required for normal signaling in taste receptor cells. Proc Natl Acad Sci U S A 115:E772–E781 [PubMed: 29311301]
27. Elliott EJ, Simon SA (1990) The anion in salt taste: a possible role for paracellular pathways. Brain Res 535:9–17 [PubMed: 1963343]
28. Finger TE, Danilova V, Barrows J, Bartel DL, Vigers AJ, Stone L, Hellekant G, Kinnamon SC (2005) ATP signaling is crucial for communication from taste buds to gustatory nerves. Science 310:1495–1499 [PubMed: 16322458]
29. Halpern BP (1998) Amiloride and vertebrate gustatory responses to NaCl. Neurosci Biobehav Rev 23:5–47 [PubMed: 9861611]
30. Heck GL, Mierson S, DeSimone JA (1984) Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. Science 223:403–405 [PubMed: 6691151]
31. Hisatsune C, Yasumatsu K, Takahashi-Iwanaga H, Ogawa N, Kuroda Y, Yoshida R, Ninomiya Y, Mikoshiba K (2007) Abnormal taste perception in mice lacking the type 3 inositol 1,4,5-trisphosphate receptor. J Biol Chem 282:37225–37231 [PubMed: 17925404]

32. Hofmann T, Chubanov V, Gudermann T, Montell C (2003) TRPM5 is a voltage-modulated and Ca^{2+}-activated monovalent selective cation channel. Curr Biol 13:1153–1158 [PubMed: 12842017]
33. Horio N, Yoshida R, Yasumatsu K, Yanagawa Y, Ishimaru Y, Matsunami H, Ninomiya Y (2011) Sour taste responses in mice lacking PKD channels. PLoS One 6:e20007 [PubMed: 21625513]
34. Huang AL, Chen X, Hoon MA, Chandrashekar J, Guo W, Trankner D, Ryba NJ, Zuker CS (2006) The cells and logic for mammalian sour taste detection. Nature 442:934–938 [PubMed: 16929298]
35. Huang YJ, Maruyama Y, Dvoryanchikov G, Pereira E, Chaudhari N, Roper SD (2007) The role of pannexin 1 hemichannels in ATP release and cell-cell communication in mouse taste buds. Proc Natl Acad Sci U S A 104:6436–6441 [PubMed: 17389364]
36. Ishimaru Y, Inada H, Kubota M, Zhuang H, Tominaga M, Matsunami H (2006) Transient receptor potential family members PKD1L3 and PKD2L1 form a candidate sour taste receptor. Proc Natl Acad Sci U S A 103:12569–12574 [PubMed: 16891422]
37. Iwatsuki K, Uneyama H (2012) Sense of taste in the gastrointestinal tract. J Pharmacol Sci 118:123–128 [PubMed: 22293296]
38. Kashio M, Wei-Qi G, Ohsaki Y, Kido MA, Taruno A (2019) CALHM1/CALHM3 channel is intrinsically sorted to the basolateral membrane of epithelial cells including taste cells. Sci Rep 9:2681 [PubMed: 30804437]
39. Kretz O, Barbry P, Bock R, Lindemann B (1999) Differential expression of RNA and protein of the three pore-forming subunits of the amiloride-sensitive epithelial sodium channel in taste buds of the rat. J Histochem Cytochem 47:51–64 [PubMed: 9857212]
40. Kusuhara Y, Yoshida R, Ohkuri T, Yasumatsu K, Voigt A, Hubner S, Maeda K, Boehm U, Meyerhof W, Ninomiya Y (2013) Taste responses in mice lacking taste receptor subunit T1R1. J Physiol 591:1967–1985 [PubMed: 23339178]
41. Larson ED, Vandenbeuch A, Voigt A, Meyerhof W, Kinnamon SC, Finger TE (2015) The role of 5-HT3 receptors in signaling from taste buds to nerves. J Neurosci 35:15984–15995 [PubMed: 26631478]
42. Lewandowski BC, Sukumaran SK, Margolskee RF, Bachmanov AA (2016) Amiloride-insensitive salt taste is mediated by two populations of type III taste cells with distinct transduction mechanisms. J Neurosci 36:1942–1953 [PubMed: 26865617]
43. Liu D, Liman ER (2003) Intracellular Ca^{2+} and the phospholipid PIP_2 regulate the taste transduction ion channel TRPM5. Proc Natl Acad Sci U S A 100:15160–15165 [PubMed: 14657398]
44. Lossow K, Hermans-Borgmeyer I, Meyerhof W, Behrens M (2020) Segregated expression of ENaC subunits in taste cells. Chem Senses 45:235–248 [PubMed: 32006019]
45. Ma J, Qi X, Yang C, Pan R, Wang S, Wu J, Huang L, Chen H, Cheng J, Wu R, Liao Y, Mao L, Wang FC, Wu Z, An JX, Wang Y, Zhang X, Zhang C, Yuan Z (2018) Calhm2 governs astrocytic ATP releasing in the development of depression-like behaviors. Mol Psychiatry 23:883–891 [PubMed: 29180673]
46. Ma Z, Saung WT, Foskett JK (2017) Action potentials and ion conductances in wild-type and CALHM1-knockout type II taste cells. J Neurophysiol 117:1865–1876 [PubMed: 28202574]
47. Ma Z, Siebert AP, Cheung KH, Lee RJ, Johnson B, Cohen AS, Vingtdeux V, Marambaud P, Foskett JK (2012) Calcium homeostasis modulator 1 (CALHM1) is the pore-forming subunit of an ion channel that mediates extracellular Ca^{2+} regulation of neuronal excitability. Proc Natl Acad Sci U S A 109:E1963–E1971 [PubMed: 22711817]
48. Ma Z, Taruno A, Ohmoto M, Jyotaki M, Lim JC, Miyazaki H, Niisato N, Marunaka Y, Lee RJ, Hoff H, Payne R, Demuro A, Parker I, Mitchell CH, Henao-Mejia J, Tanis JE, Matsumoto I, Tordoff MG, Foskett JK (2018) CALHM3 is essential for rapid ion channel-mediated purinergic neurotransmission of GPCR-mediated tastes. Neuron 98:547–561 [PubMed: 29681531]
49. Matsumoto I, Emori Y, Nakamura S, Shimizu K, Arai S, Abe K (2003) DNA microarray cluster analysis reveals tissue similarity and potential neuron-specific genes expressed in cranial sensory ganglia. J Neurosci Res 74:818–828 [PubMed: 14648586]
50. McCutcheon NB (1992) Human psychophysical studies of saltiness suppression by amiloride. Physiol Behav 51:1069–1074 [PubMed: 1615046]

51. Medler KF, Margolskee RF, Kinnamon SC (2003) Electrophysiological characterization of voltage-gated currents in defined taste cell types of mice. J Neurosci 23:2608–2617 [PubMed: 12684446]
52. Miyamoto T, Miyazaki T, Okada Y, Sato T (1996) Whole-cell recording from non-dissociated taste cells in mouse taste bud. J Neurosci Methods 64:245–252 [PubMed: 8699887]
53. Moyer BD, Hevezi P, Gao N, Lu M, Kalabat D, Soto H, Echeverri F, Laita B, Yeh SA, Zoller M, Zlotnik A (2009) Expression of genes encoding multi-transmembrane proteins in specific primate taste cell populations. PLoS One 4:e7682 [PubMed: 19997627]
54. Mozaffarian D, Fahimi S, Singh GM, Micha R, Khatibzadeh S, Engell RE, Lim S, Danaei G, Ezzati M, Powles J, Global Burden of Diseases N, Chronic Diseases Expert G (2014) Global sodium consumption and death from cardiovascular causes. N Engl J Med 371:624–634 [PubMed: 25119608]
55. Murata Y, Yasuo T, Yoshida R, Obata K, Yanagawa Y, Margolskee RF, Ninomiya Y (2010) Action potential-enhanced ATP release from taste cells through hemichannels. J Neurophysiol 104:896–901 [PubMed: 20519578]
56. Murtaza B, Hichami A, Khan AS, Ghiringhelli F, Khan NA (2017) Alteration in taste perception in cancer: causes and strategies of treatment. Front Physiol 8:134 [PubMed: 28337150]
57. Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJ, Zuker CS (2002) An amino-acid taste receptor. Nature 416:199–202 [PubMed: 11894099]
58. Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, Zuker CS (2001) Mammalian sweet taste receptors. Cell 106:381–390 [PubMed: 11509186]
59. Ninomiya Y (1998) Reinnervation of cross-regenerated gustatory nerve fibers into amiloride-sensitive and amiloride-insensitive taste receptor cells. Proc Natl Acad Sci U S A 95:5347–5350 [PubMed: 9560278]
60. Nomura K, Nakanishi M, Ishidate F, Iwata K, Taruno A (2020) All-electrical Ca^{2+}-independent signal transduction mediates attractive sodium taste in taste buds. Neuron 106:816–829 [PubMed: 32229307]
61. Noreng S, Bharadwaj A, Posert R, Yoshioka C, Baconguis I (2018) Structure of the human epithelial sodium channel by cryo-electron microscopy. Elife 7:e39340 [PubMed: 30251954]
62. Oka Y, Butnaru M, von Buchholtz L, Ryba NJ, Zuker CS (2013) High salt recruits aversive taste pathways. Nature 494:472–475 [PubMed: 23407495]
63. Ossebaard CA, Smith DV (1995) Effect of amiloride on the taste of NaCl, Na-gluconate and KCl in humans: implications for Na^+ receptor mechanisms. Chern Senses 20:37–46
64. Ossebaard CA, Smith DV (1996) Amiloride suppresses the sourness of NaCl and LiCl. Physiol Behav 60:1317–1322 [PubMed: 8916188]
65. Perez CA, Huang L, Rong M, Kozak JA, Preuss AK, Zhang H, Max M, Margolskee RF (2002) A transient receptor potential channel expressed in taste receptor cells. Nat Neurosci 5:1169–1176 [PubMed: 12368808]
66. Prawitt D, Monteilh-Zoller MK, Brixel L, Spangenberg C, Zabel B, Fleig A, Penner R (2003) TRPM5 is a transient Ca^{2+}-activated cation channel responding to rapid changes in $[Ca^{2+}]_i$. Proc Natl Acad Sci U S A 100:15166–15171 [PubMed: 14634208]
67. Ren Y, Wen T, Xi Z, Li S, Lu J, Zhang X, Yang X, Shen Y (2020) Cryo-EM structure of the calcium homeostasis modulator 1 channel. Sci Adv 6:eaba8161 [PubMed: 32832630]
68. Richter TA, Caicedo A, Roper SD (2003) Sour taste stimuli evoke Ca^{2+} and pH responses in mouse taste cells. J Physiol 547:475–483 [PubMed: 12562903]
69. Richter TA, Dvoryanchikov GA, Roper SD, Chaudhari N (2004) Acid-sensing ion channel-2 is not necessary for sour taste in mice. J Neurosci 24:4088–4091 [PubMed: 15102924]
70. Roebber JK, Roper SD, Chaudhari N (2019) The role of the anion in salt (NaCl) detection by mouse taste buds. J Neurosci 39:6224–6232 [PubMed: 31171579]
71. Romanov RA, Bystrova MF, Rogachevskaya OA, Sadovnikov VB, Shestopalov VI, Kolesnikov SS (2012) The ATP permeability of pannexin 1 channels in a heterologous system and in mammalian taste cells is dispensable. J Cell Sci 125:5514–5523 [PubMed: 22956545]
72. Romanov RA, Lasher RS, High B, Savidge LE, Lawson A, Rogachevskaja OA, Zhao H, Rogachevsky VV, Bystrova MF, Churbanov GD, Adameyko I, Harkany T, Yang R, Kidd GJ, Marambaud P, Kinnamon JC, Kolesnikov SS, Finger TE (2018) Chemical synapses without

synaptic vesicles: purinergic neurotransmission through a CALHM1 channel-mitochondrial signaling complex. Sci Signal 11:eaao 1815

73. Romanov RA, Rogachevskaja OA, Bystrova MF, Jiang P, Margolskee RF, Kolesnikov SS (2007) Afferent neurotransmission mediated by hemichannels in mammalian taste cells. EMBO J 26:657–667 [PubMed: 17235286]
74. Romanov RA, Rogachevskaja OA, Khokhlov AA, Kolesnikov SS (2008) Voltage dependence of ATP secretion in mammalian taste cells. J Gen Physiol 132:731–744 [PubMed: 19029378]
75. Roper SD (2015) The taste of table salt. Pflugers Arch 467:457–463 [PubMed: 25559847]
76. Saotome K, Teng B, Tsui CCA, Lee WH, Tu YH, Kaplan JP, Sansom MSP, Liman ER, Ward AB (2019) Structures of the otopetrin proton channels Otopl and Otop3. Nat Struct Mol Biol 26:518–525 [PubMed: 31160780]
77. Schiffman SS, Lockhead E, Maes FW (1983) Amiloride reduces the taste intensity of Na^+ and Li^+ salts and sweeteners. Proc Natl Acad Sci U S A 80:6136–6140 [PubMed: 6577473]
78. Sclafani A, Ackroff K (2018) Greater reductions in fat preferences in CALHM1 than CD36 knockout mice. Am J Phys Regul Integr Comp Phys 315:R576–R585
79. Shigemura N, Ninomiya Y (2016) Recent advances in molecular mechanisms of taste signaling and modifying. Int Rev Cell Mol Biol 323:71–106 [PubMed: 26944619]
80. Siebert AP, Ma Z, Grevet JD, Demuro A, Parker I, Foskett JK (2013) Structural and functional similarities of calcium homeostasis modulator 1 (CALHM1) ion channel with connexins, pannexins, and innexins. J Biol Chem 288:6140–6153 [PubMed: 23300080]
81. Smith DV, Ossebaard CA (1995) Amiloride suppression of the taste intensity of sodium chloride: evidence from direct magnitude scaling. Physiol Behav 57:773–777 [PubMed: 7777616]
82. Stähler F, Riedel K, Demgensky S, Neumann K, Dunkel A, Täubert A, Raab B, Behrens M, Raguse JD, Hofmann T, Meyerhof W (2008) A role of the epithelial sodium channel in human salt taste transduction? Chemosens Percept 1:78–90
83. Stevens DR, Seifert R, Bufe B, Muller F, Kremmer E, Gauss R, Meyerhof W, Kaupp UB, Lindemann B (2001) Hyperpolarization-activated channels HCN1 and HCN4 mediate responses to sour stimuli. Nature 413:631–635 [PubMed: 11675786]
84. Strazzullo P, D'Elia L, Kandala NB, Cappuccio FP (2009) Salt intake, stroke, and cardiovascular disease: meta-analysis of prospective studies. BMJ 339:b4567 [PubMed: 19934192]
85. Syrjanen JL, Michalski K, Chou TH, Grant T, Rao S, Simorowski N, Tucker SJ, Grigorieff N, Furukawa H (2020) Structure and assembly of calcium homeostasis modulator proteins. Nat Struct Mol Biol 27:150–159 [PubMed: 31988524]
86. Tan HE, Sisti AC, Jin H, Vignovich M, Villavicencio M, Tsang KS, Goffer Y, Zuker CS (2020) The gut-brain axis mediates sugar preference. Nature 580:511–516 [PubMed: 32322067]
87. Tanis JE, Ma Z, Krajacic P, He L, Foskett JK, Lamitina T (2013) CLHM-1 is a functionally conserved and conditionally toxic Ca^{2+}-pemreable ion channel in Caenorhabditis elegans. J Neurosci 33:12275–12286 [PubMed: 23884934]
88. Taruno A (2018) ATP Release Channels. Int J Mol Sci 19:808
89. Taruno A, Matsumoto I, Ma Z, Marambaud P, Foskett JK (2013) How do taste cells lacking synapses mediate neurotransmission? CALHM1, a voltage-gated ATP channel. Bioessays 35:1111–1118 [PubMed: 24105910]
90. Taruno A, Sun H, Nakajo K, Murakami T, Ohsaki Y, Kido MA, Ono F, Marunaka Y (2017) Post-translational palmitoylation controls the voltage gating and lipid raft association of the CALHM1 channel. J Physiol 595:6121–6145 [PubMed: 28734079]
91. Taruno A, Vingtdeux V, Ohmoto M, Ma Z, Dvoryanchikov G, Li A, Adrien L, Zhao H, Leung S, Abernethy M, Koppel J, Davies P, Civan MM, Chaudhari N, Matsumoto I, Hellekant G, Tordoff MG, Marambaud P, Foskett JK (2013) CALHM1 ion channel mediates purinergic neurotransmission of sweet, bitter and umami tastes. Nature 495:223–226 [PubMed: 23467090]
92. Teng B, Wilson CE, Tu YH, Joshi NR, Kinnamon SC, Liman ER (2019) Cellular and neural responses to sour stimuli require the proton channel Otop1. Curr Biol 29:3647–3656 e3645 [PubMed: 31543453]
93. Tennissen AM (1992) Amiloride reduces intensity responses of human fungiform papillae. Physiol Behav 51:1061–1068 [PubMed: 1615045]

94. Tennissen AM, McCutcheon NB (1996) Anterior tongue stimulation with amiloride suppresses NaCl saltiness, but not citric acid sourness in humans. Chem Senses 21:113–120 [PubMed: 8670690]
95. Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D (1998) The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21:531–543 [PubMed: 9768840]
96. Tordoff MG, Aleman TR, Ellis HT, Ohmoto M, Matsumoto I, Shestopalov VI, Mitchell CH, Foskett JK, Poole RL (2015) Normal taste acceptance and preference of PANX1 knockout mice. Chem Senses 40:453–459 [PubMed: 25987548]
97. Tordoff MG, Ellis HT, Aleman TR, Downing A, Marambaud P, Foskett JK, Dana RM, McCaughey SA (2014) Salty taste deficits in CALHM1 knockout mice. Chem Senses 39:515–528 [PubMed: 24846212]
98. Tu YH, Cooper AJ, Teng B, Chang RB, Artiga DJ, Turner HN, Muthall EM, Ye W, Smith AD, Liman ER (2018) An evolutionarily conserved gene family encodes proton-selective ion channels. Science 359:1047–1050 [PubMed: 29371428]
99. Ugawa S, Minami Y, Guo W, Saishin Y, Takatsuji K, Yamamoto T, Tohyama M, Shimada S (1998) Receptor that leaves a sour taste in the mouth. Nature 395:555–556 [PubMed: 9783580]
100. Vandenbeuch A, Anderson CB, Kinnamon SC (2015) Mice lacking pannexin 1 release ATP and respond normally to all taste qualities. Chem Senses 40:461–467 [PubMed: 26136251]
101. Vandenbeuch A, Anderson CB, Parnes J, Enjyoji K, Robson SC, Finger TE, Kinnamon SC (2013) Role of the ectonucleotidase NTPDase2 in taste bud function. Proc Natl Acad Sci U S A 110: 14789–14794 [PubMed: 23959882]
102. Vandenbeuch A, Clapp TR, Kinnamon SC (2008) Amiloride-sensitive channels in type I fungiform taste cells in mouse. BMC Neurosci 9:1 [PubMed: 18171468]
103. Vandenbeuch A, Zorec R, Kinnamon SC (2010) Capacitance measurements of regulated exocytosis in mouse taste cells. J Neurosci 30:14695–14701 [PubMed: 21048127]
104. Waldmann R, Champigny G, Bassilana F, Voilley N, Lazdunski M (1995) Molecular cloning and functional expression of a novel amiloride-sensitive Na^+ channel. J Biol Chem 270:27411–27414 [PubMed: 7499195]
105. Wang YY, Chang RB, Allgood SD, Silver WL, Liman ER (2011) A TRPA1-dependent mechanism for the pungent sensation of weak acids. J Gen Physiol 137:493–505 [PubMed: 21576376]
106. Wilson CE, Vandenbeuch A, Kinnamon SC (2019) Physiological and behavioral responses to optogenetic stimulation of $PKD2L1^+$ type III taste cells. eNeuro 6:ENEURO.0107-19.2019
107. Yang W, Wang Y, Guo J, He L, Zhou Y, Zheng H, Liu Z, Zhu P, Zhang XC (2020) Cryo-electron microscopy structure of CLHM1 ion channel from Caenorhabditis elegans. Protein Sci 29:1803–1815 [PubMed: 32557855]
108. Yasumatsu K, Manabe T, Yoshida R, Iwatsuki K, Uneyama H, Takahashi I, Ninomiya Y (2015) Involvement of multiple taste receptors in umami taste: analysis of gustatory nerve responses in metabotropic glutamate receptor 4 knockout mice. J Physiol 593:1021–1034 “ [PubMed: 25529865]
109. Yasumatsu K, Ohkuri T, Yoshida R, Iwata S, Margolskee RF, Ninomiya Y (2020) Sodium-glucose cotransporter 1 as a sugar taste sensor in mouse tongue. Acta Physiol (Oxf):e13529 [PubMed: 32599649]
110. Ye W, Chang RB, Bushman JD, Tu YH, Mulhall EM, Wilson CE, Cooper AJ, Chick WS, Hill-Eubanks DC, Nelson MT, Kinnamon SC, Liman ER (2016) The K^+ channel KIR2.1 functions in tandem with proton influx to mediate sour taste transduction. Proc Natl Acad Sci U S A 113:E229–E238 [PubMed: 26627720]
111. Yee KK, Sukumaran SK, Kotha R, Gilbertson TA, Margolskee RF (2011) Glucose transporters and ATP-gated K^+ (K_{ATP}) metabolic sensors are present in type 1 taste receptor 3 (T1r3(-expressing taste cells. Proc Natl Acad Sci U S A 108:5431–5436 [PubMed: 21383163]
112. Yoshida R, Horio N, Murata Y, Yasumatsu K, Shigemura N, Ninomiya Y (2009) NaCl responsive taste cells in the mouse fungiform taste buds. Neuroscience 159:795–803 [PubMed: 19167465]

113. Yoshida R, Shigemura N, Sanematsu K, Yasumatsu K, Ishizuka S, Ninomiya Y (2006) Taste responsiveness of fungiform taste cells with action potentials. J Neurophysiol 96:3088–3095 [PubMed: 16971686]

114. Zhang J, Jin H, Zhang W, Ding C, O'Keeffe S, Ye M, Zuker CS (2019) Sour sensing from the tongue to the brain. Cell 179:392–402 [PubMed: 31543264]

115. Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, Zuker CS, Ryba NJ (2003) Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 112:293–301 [PubMed: 12581520]

116. Zhang Z, Zhao Z, Margolskee R, Liman E (2007) The transduction channel TRPM5 is gated by intracellular calcium in taste cells. J Neurosci 27:5777–5786 [PubMed: 17522321]

117. Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NJ, Zuker CS (2003) The receptors for mammalian sweet and umami taste. Cell 115:255–266 [PubMed: 14636554]

118. Zocchi D, Wennemuth G, Oka Y (2017) The cellular mechanism for water detection in the mammalian taste system. Nat Neurosci 20:927–933 [PubMed: 28553944]

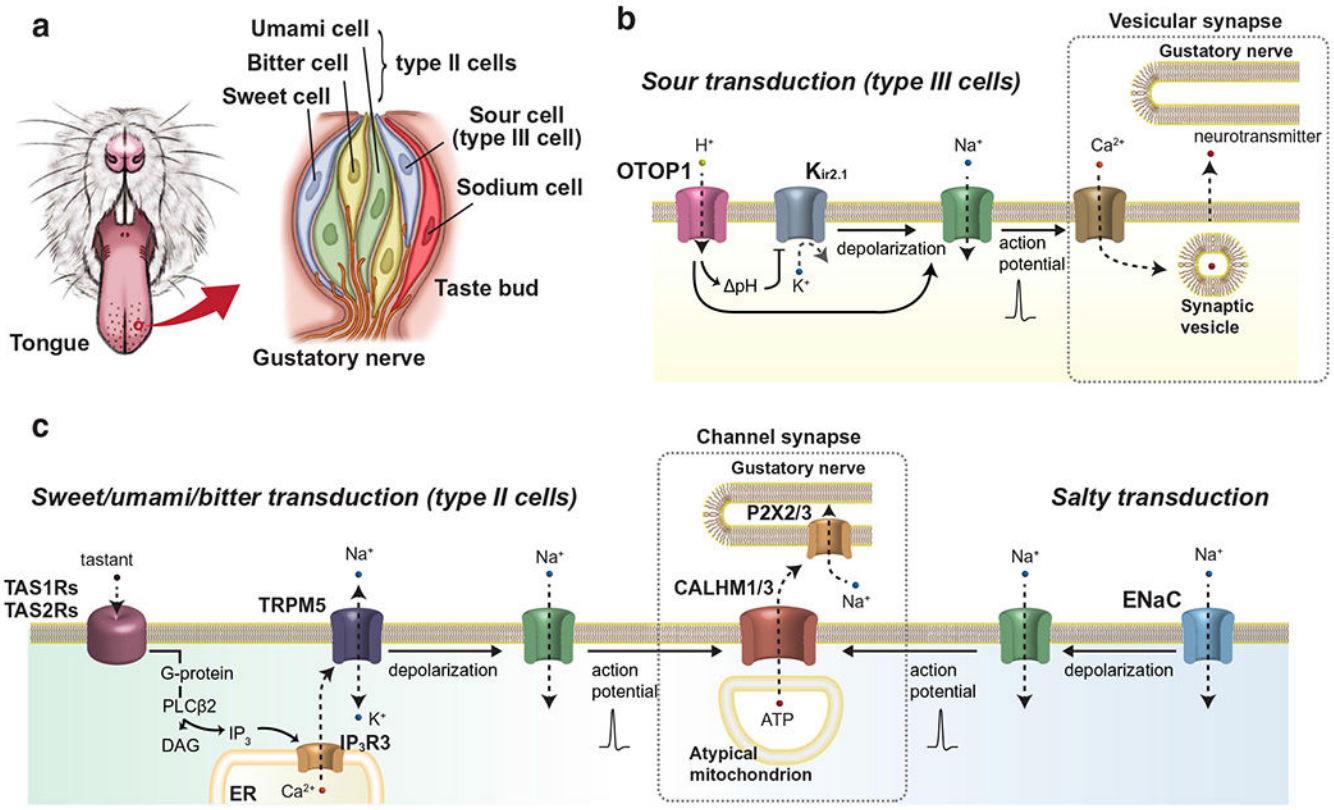

Fig. 1.

Signal transduction and neurotransmission of tastes. **a** Taste coding in taste buds. Each taste quality is generally sensed by dedicated taste cells: sweet, bitter, umami, sour, and sodium cells. Whereas sour taste cells are designated as type III cells, sweet, bitter, and umami taste cells are considered together as type II cells because they share a common signaling cascade. Note that high-salt taste relies on bitter and sour cells. **b** Sour transduction in type III cells involves OTOP1 and $K_{ir2.1}$ as the H^+ sensor and signal amplifier channels, respectively, and employs conventional vesicular synapses for neurotransmission. **c** Among type II cells, sweet, umami, and bitter cells differ in the types of taste receptors but share a similar signaling pathway, where the activation of TRPM5 channels generates a depolarization to trigger action potential firing. Sodium taste transduction is initiated by Na^+ influx through ENaC, which induces a supra-threshold depolarization for action potential generation. Both type II cells and sodium taste cells employ the channel synapse involving CALHM1/3 channels as the conduit for action potential-dependent neurotransmitter release. OTOP1, Otopetrin1; $K_{ir2.1}$, inward rectifier K^+ channel; ΔpH, intracellular acidification; PLCβ2, phospholipase Cβ2; IP_3, inositol trisphosphate; DAG, diacylglycerol; ER, endoplasmic reticulum; IP_3R3, inositol trisphosphate receptor type 3; TRPM, transient receptor potential melastatin; CALHM, calcium homeostasis modulator; ENaC, epithelial Na^+ channel

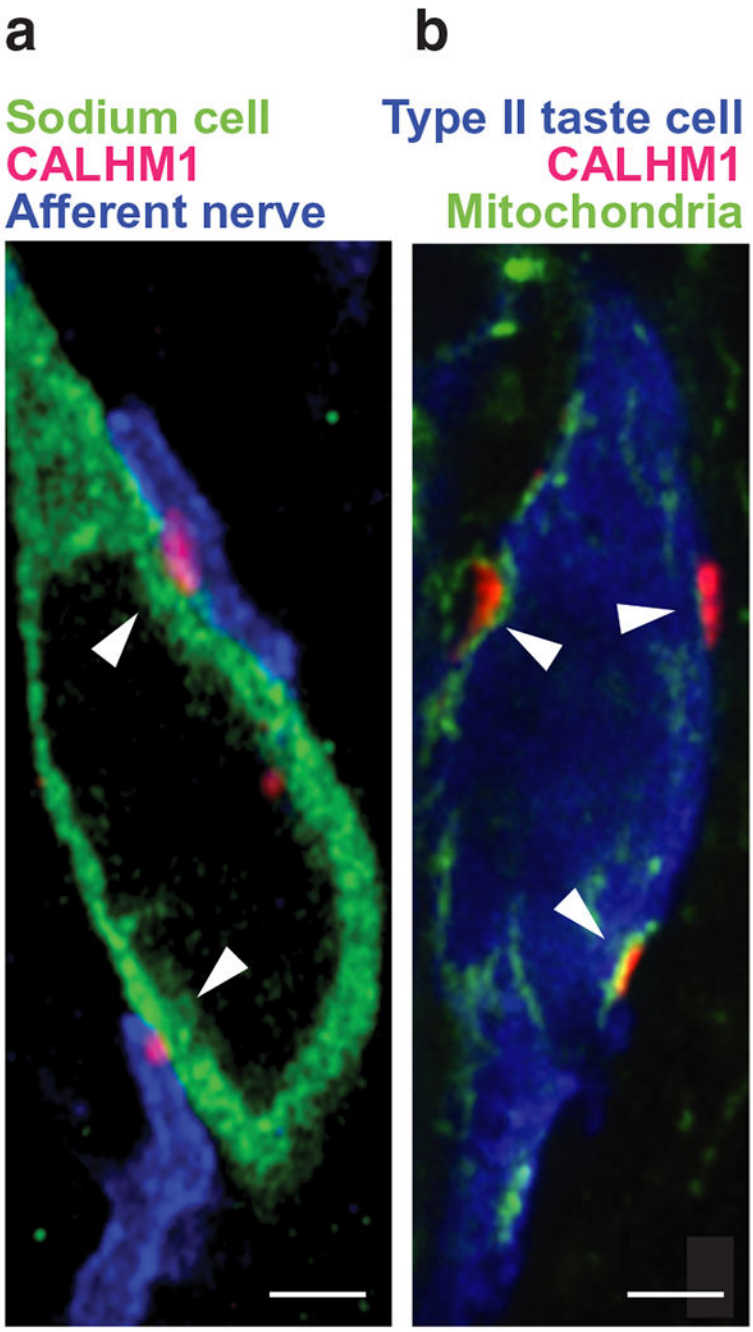

Fig. 2.

Structure of the channel synapse defined by characteristic localization of CALHM channels, afferent nerve fibers, and mitochondria. **a** Super-resolution image of a sodium taste cell identified by expression of GCaMP3 (green) with co-staining of CALHM1 (red) and an afferent nerve fiber (P2X2, blue) in a fungiform taste bud of an ENaCα-GCaMP3 mouse. Data were taken from [60]. **b** Super-resolution image of a type II taste cell identified by expression of PLCβ2 (blue) with co-staining of CALHM1 (red) and mitochondria (cytochrome c, green) in a circumvallate taste bud of a B6 mouse. Arrowheads indicate

channel synapses where mitochondria, CALHM1, and afferent nerves localize closely together. Scale bars, 2 μm

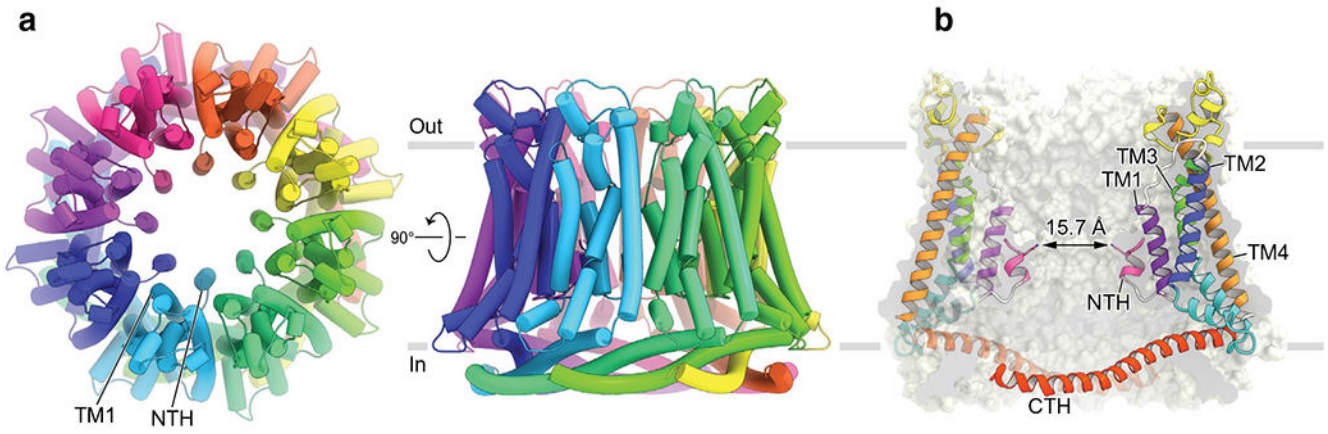

Fig. 3.

Structure of the CALHM1 channel. **a** Overall structure of the killifish CALHM1 octamer (PDB ID: 6LMT), viewed from the extracellular side (left) and parallel to the membrane (right). **b** Channel pore of killifish CALHM1, shown in cross section of the surface representation. Each region for two opposing subunits is colored as follows: NTH, pink; TM1, purple; TM2, blue; TM3, light green; TM4, orange; extracellular region, yellow; parts of TM2 and TM3 at the intracellular side, cyan; and CTH, red