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Opening a "Wide" Window onto Taste Signal Transmission

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Abstract

Taste bud cells for sweet, umami, and bitter transmit sensory signals without a synapse. A study by Ma et al. (2018) finds a key ATP-permeable pore-forming subunit required for rapid neurotransmission from the tongue to secondary taste neurons.

Peripheral sensory neurons generally transmit sensory information to higher-order neurons via synaptic vesicle release. By contrast, some types of mature taste bud cells (TBCs) are known to lack synaptic structures, yet they can transmit taste signals. For more than two decades, sensory biologists have struggled to unveil how synapse-less TBCs communicate with downstream neurons. On the mammalian tongue, each taste bud contains several dozens of TBCs that are classified into receptor cells and support cells (sometimes referred to as type I cells). Individual taste receptor cells are uniquely tuned to mediate one of five basic tastes (Yarmolinsky et al., 2009). Notably, TBCs lack axons and are innervated by the afferent nerve fibers of secondary taste neurons (Figure 1, top).

Interestingly, acid-sensing TBCs (type III cells) have neuronal properties with visible synaptic structures (Kataoka et al., 2008), whereas sweet-, umami-, and bitter-sensing cells (type II cells) do not possess the synaptic machinery (Clapp et al., 2004). How are sweet, umami, and bitter signals transmitted from taste buds? An important clue came from a study on neurotransmitter molecules. Finger et al. (2005) found that TBCs use ATP as a critical transmitter to communicate with gustatory nerve fibers. Knocking out purinergic receptors P2X2 and P2X3 completely eliminated physiological responses to all tastants. Since P2X2 and P2X3 are the major purinergic receptors in secondary taste neurons, this study implicated that sweet, umami, and bitter TBCs somehow secrete ATP without synaptic vesicles.

ATP can be released from cells via vesicular as well as non-vesicular routes, including certain types of ATP-permeable channels (Kinnamon, 2013). If such channels are responsible for neurotransmission in TBCs, they need to meet at least three criteria. First, the channels obviously have to be expressed in TBCs. Second, knocking out the gene should eliminate ATP release from TBCs without affecting cell excitability and viability. Third, such genetic manipulation should abolish physiological and behavioral responses to sweet, umami, and bitter taste stimuli. In the past 10 years, multiple channels have been proposed

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as ATP-conducting molecules, including pannexin 1 (Huang et al., 2007) and connexins (Romanov et al., 2007). However, their roles in neurotransmission are still elusive.

In 2013, Taruno et al. (2013) discovered that calcium homeostasis modulator 1 (CALHM1 or FAM26C), the pore-forming subunit of a voltage-gated, non-selective cation channel, is selectively expressed in type II TBCs. This large-pore channel seems to meet all the criteria mentioned above for ATP-conducting molecules in taste buds. Animals lacking the CALHM1 gene (*CALHM1*$^{-/-}$ mice) showed no taste-induced ATP release from taste tissues. Moreover, these mice lost behavioral preference for sweet, umami, and bitter without their responses to salty or sour tastes being affected. Importantly, type II TBCs retained normal voltage-gated Na^+ currents as well as calcium responses upon taste stimulation in the absence of CALMH1. Data from this study led to the conclusion that CALHM1 is a key component in forming an ATP-permeable channel in type II TBCs.

Despite the compelling evidence that CALHM1 is involved in ATP release from TBCs, a critical issue remained unsolved. The kinetics of ATP release through CALHM1 monomeric channels is unrealistically slow for biological taste responses (τ > 500 msec). Generally, taste cells generate action potentials upon taste stimulation, with the latency of ATP release being ~10 msec. This obvious temporal discrepancy suggested that an additional component(s) is required for rapid ATP release from TBCs.

In this issue of Neuron, Ma et al. (2018) identified a key channel subunit that, together with CALHM1, forms the endogenous ATP-permeable channel complex in Type II TBCs. For a potential partner of CALHM1, the authors focused on the members of the CALHM family, *CALHM2* (FAM26B) and *CALHM3* (FAM26A). Using electrophysiological recording in heterologous expression systems, they found that co-expression of CALHM1 with CALHM3, but not with CALHM2, drastically enhances the activation kinetics of outward currents (presumably induced by ATP release) compared to expression of CALHM1 alone. In fact, cells transfected with CALHM1+3 exhibited gating kinetics similar to the kinetics in intact TBCs. Given these results, the authors hypothesized that CALHM1 and CALHM3 form a complex to mediate rapid taste neurotransmission.

To test this idea, Ma et al. (2018) performed a series of biochemical experiments. First, they showed that CALHM1 and CALHM3 physically and specifically interact with each other by co-immunoprecipitation in culture cells. Second, the authors used various CALHM1/3 concatemers to determine that these two subunits likely form a hexametric channel complex. Third, they employed a single-molecule photobleaching technique and showed that bleaching steps of individual, fluorescently tagged subunits (as an estimation of the number of subunits) decreased in the presence of the partner subunit. This suggests that CALHM1 and CALHM3 assemble into a single channel complex. These biochemical studies also revealed one unexpected pharmacological property of CALHM channels. The application of carbenoxolone (CBX), a nonselective gap-junction blocker, has been reported to suppress ATP release from TBCs (Huang et al., 2007). Previous studies used this evidence to support the contribution of gap-junction proteins to taste neurotransmission. Remarkably, while CBX has no inhibitory effect on CALHM1 homomeric channels, the authors found that CALHM1+3 heteromeric channels are highly sensitive to CBX. These results, at least in

part, explain the inhibitory effect of the gap junction blocker on ATP release from intact TBCs.

So the next important question is whether CALHM1+3 indeed functions as the endogenous, voltage-gated ATP-release channel *in vivo*. Ma et al. (2018) first demonstrated that these two subunits are co-expressed in TBCs that also express TRPM5, a cation channel required for sweet, umami, and bitter tastes. Next, they found that voltage-gated nonselective outward currents were absent in type II TBCs isolated from *CALHM3*$^{-/-}$ mice. However, such genetic deletion did not alter taste-evoked calcium signaling in these cells. Finally, they examined the behavioral taste preference using these knockout mice. Consistent with their hypothesis, *CALHM3*$^{-/-}$ mice exhibited practically no electrophysiological responses and exhibited severely reduced behavioral preference toward GPCR-mediated tastes (i.e., sweet, umami, and bitter).

This study by Ma et al. (2018) elegantly demonstrated that CALHM1+3 channels form voltage-dependent cation channels in type II cells and are required for rapid ATP release. There are a few caveats, though, in their interpretations and conclusions. For instance, the ATP permeability of the channel complex has not been directly tested in this study. This leaves the possibility that effector molecules downstream of CALHM1+3 channels may contribute to ATP release. Another issue has to do with technical difficulties: obtaining workable antibodies for ion channels and sufficient amounts of protein from TBCs are notoriously challenging. Due to these limitations, the direct interaction between CALHM1 and CALHM3 has been tested only in a heterologous overexpression system instead of in intact TBCs. Notably, the authors examined aversive salt responses (high salt) but not attractive salt responses (amiloride-sensitive low salt) in *CALHM3*$^{-/-}$ mice. Since these two aspects of salt taste are mediated by distinct pathways, attractive salt taste may also be affected in *CALHM3*$^{-/-}$ mice.

Identification of a critical component for ATP transmission in taste buds addresses a long-standing question in the field. However, it also opens up a new avenue to tackle important problems to be solved in the future. Taste buds are densely packed with functionally distinct TBCs, each of which is innervated by secondary taste neurons in a quality-specific manner (Lee et al., 2017). How does a given nerve fiber selectively respond to ATP release from the target TBC, but not from adjacent cells? Possible mechanisms are: 1) ATP-permeable channels involving CALHM1+3 may be localized to the sites of taste nerve ending, and 2) the open probability of the channels may be locally regulated by additional cellular components. In either scenario, deciphering the underlying molecular basis would provide further insight for purinergic transmission in the taste system. Another remaining question is the interaction between CALHM and other ATP-permeable channels. For example, pannexin 1 is highly expressed in TBCs and is capable of releasing ATP, whereas gene deletion has no effect on taste responses. It is feasible that these molecules may have a role for cell-to-cell communication between TBCs. This model would indicate that ATP is used for both neurotransmission and intra-taste-bud signaling via distinct ATP-release mechanisms. Further studies would clarify the contribution of each ATP-permeable channel to different aspects of physiological functions mediated by ATP. So far, two neurotransmission mechanisms have been found in the peripheral taste system (Figure 1). Acid-sensing TBCs

are “neuronal” cells with visible synaptic vesicles that secrete ATP upon stimulation. Sweet, umami, and bitter TBCs are “non-neuronal” cell types that release ATP via CALHM channels. How about salty? It seems that salt-sensing TBCs also use ATP as a neurotransmitter because knocking out of P2X2 and P2X3 eliminates salt responses (Finger et al., 2005). However, the route of ATP release is unclear. Understanding how salt-sensing cells transmit signals to secondary taste neurons would put the final piece in the puzzle of taste neurotransmission.

The important roles of purinergic signaling in neuromodulation are widely accepted at both peripheral and central levels. In this study, Ma et al. (2018) provide the first functional evidence of ion-channel-mediated fast purinergic neurotransmission. Whether this mechanism is unique to the taste system or more broadly used in other systems remains to be investigated.

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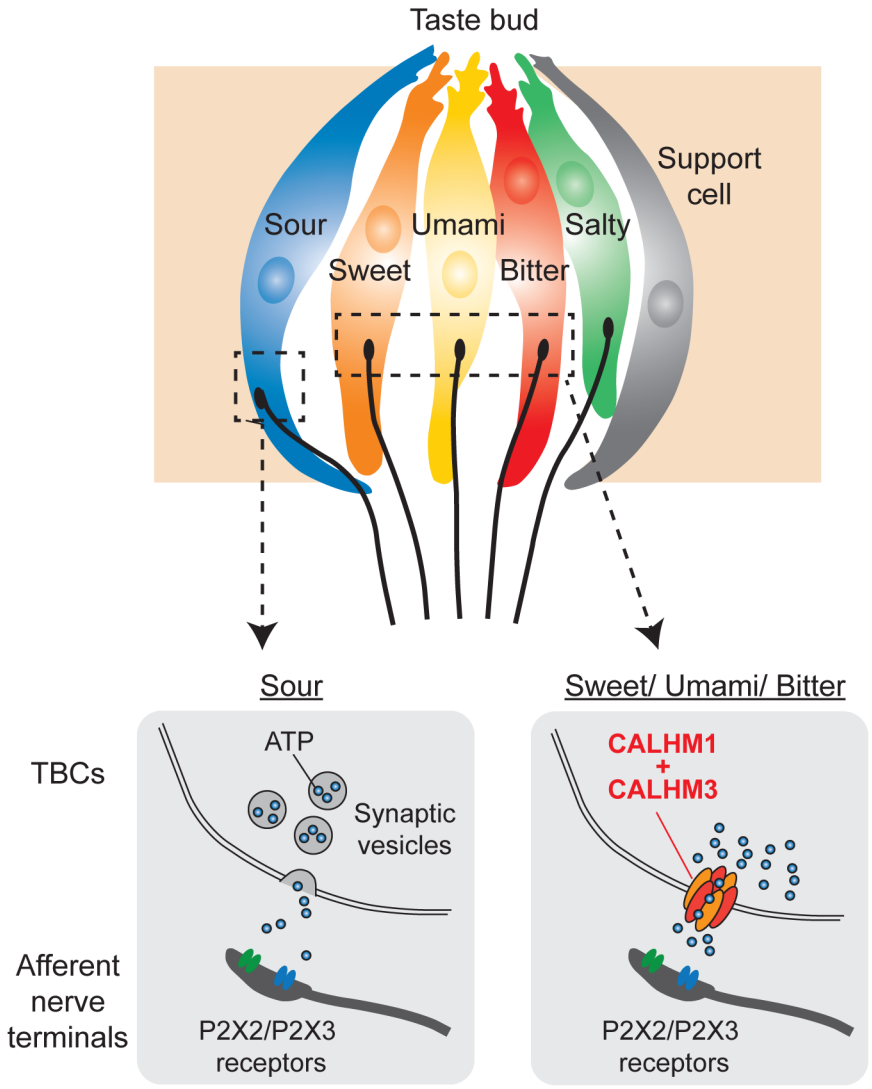

Figure 1. Purinergic Neurotransmission of Taste Bud Cells

Schematic diagram illustrating that taste bud cells (TBCs) are innervated by afferent nerve fibers from secondary taste neurons (top). Individual TBCs are tuned to mediate one of five basic tastes. Based on morphological characteristics, support cells are sometimes termed type I; sweet, umami, and bitter cells are type II; and sour cells are type III TBCs. Once TBCs receive taste stimulation, they generate action potentials leading to ATP release, which in turn activates P2X2 and P2X3 receptors expressed by afferent nerve fibers. Sour-sensing TBCs use conventional synaptic vesicles to secrete ATP (bottom left), whereas sweet, umami, and bitter TBCs release ATP via CALHM1/CALHM3 channels (bottom right).