TNFα delivery (Figs. 4D and 1B), and TACE, the enzyme responsible for cleavage and release of soluble TNFα (22) (Fig. 4D). Lastly, we observed the movement of GFP-TNFα to the phagocytic cup during the internalization of C. albicans in live cells (Fig. 4E and movies S4 and S5). Membranes containing GFP-TNFα or GFP-VAMP3 were seen forming the initial phagocytic cup. As membrane moved around the yeast, engulfing and internalizing it, TNFα was concentrated in a patch on the outermost point of the cell surface from where it was cleaved and released (Fig. 4E, fig. S3, and movies S4 and S5). Thus, TNFα- and VAMP3-containing RE membranes translocate to the nascent phagocytic cup for SNARE-mediated fusion during the initial stages of phagocytosis. This presents both an unexpected site for cytokine secretion and a rapid and efficient mechanism for release of an early response inflammatory mediator. Having a single route for membrane flow to the cell surface via the RE that both delivers TNFα to the plasma membrane and expands the plasma membrane for phagocytic cup formation neatly combines two early actions of activated macrophages mounting an innate immune response.

References and Notes

ATP Signaling Is Crucial for Communication from Taste Buds to Gustatory Nerves
Thomas E. Finger,1,2 Vicktoria Danilova,3 Jennell Barrows,1,2 Dianna L. Bartel,1,2 Alison J. Vigers,1,2 Leslie Stone,1,4 Goran Hellekant,3,5 Sue C. Kinnamon1,4,6

Taste receptor cells detect chemicals in the oral cavity and transmit this information to taste nerves, but the neurotransmitter(s) have not been identified. We report that adenosine 5′-triphosphate (ATP) is the key neurotransmitter in this system. Genetic elimination of ionotropic purinergic receptors (P2X and P2X) eliminates taste responses in the taste nerves, although the nerves remain responsive to touch, temperature, and menthol. Similarly, P2X-knockout mice show greatly reduced behavioral responses to sweeteners, glutamate, and bitter substances. Finally, stimulation of taste buds in vivo evokes release of ATP. Thus, ATP fulfils the criteria for a neurotransmitter linking taste buds to the nervous system.

Taste buds transduce chemical signals in the mouth into neural messages transmitted to gustatory nerve fibers of the facial and glossopharyngeal nerves. Despite recent progress in delineating the molecular mechanisms for taste transduction, the identity of the neurotransmitter from taste buds to the nerve fibers is unknown.

1Rocky Mountain Taste and Smell Center, 2University of Colorado School of Medicine, Aurora CO 80045, USA, 3Department of Animal Health and Biomedical Science, University of Wisconsin, Madison, WI 53706, USA, 4Department of Biomedical Sciences, Colorado State University, Fort Collins, CO 80523, USA, 5Departments of Physiology and Pharmacology, University of Minnesota, Duluth, MN 55812, USA.
*To whom correspondence should be addressed. E-mail: sue.kinnamon@colostate.edu

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23. Materials and methods and supporting data are available on Science Online.
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not responsive to taste. These findings indicate that P2X<sub>2</sub> and/or P2X<sub>3</sub> receptors are essential for activation of the gustatory nerves.

To characterize taste-related behaviors of the P2X<sub>2</sub>/P2X<sub>3</sub><sup>Dbl<sup>-/-</sup></sup> (KO) mice, two-bottle preference tests were used (10). Compared with P2X<sub>2</sub>/P2X<sub>3</sub><sup>Dbl<sup>+/+</sup></sup> (WT) mice, the P2X<sub>2</sub>/P2X<sub>3</sub><sup>Dbl<sup>-/-</sup></sup> (KO) mice were not responsive to artificial sweeteners (Fig. 2, A and C), sucrose (Fig. 2B), or monosodium glutamate (Fig. 2E) and denatonium benzoate (Fig. 2F). Single-KO mice, lacking either the P2X<sub>2</sub> or P2X<sub>3</sub> subunit, also were assessed in two-bottle preference tests for certain key tastants. The P2X<sub>2</sub>-only KO animals were significantly impaired in their responses to SC45647, saccharin, and denatonium (fig. S2), whereas the P2X<sub>3</sub>-only KO mice were only marginally impaired to denatonium. The single-KO strains were, however, at least an order of magnitude more responsive than the P2X<sub>2</sub>/P2X<sub>3</sub><sup>Dbl<sup>-/-</sup></sup> (KO) line. Thus, loss of either P2X<sub>2</sub> or P2X<sub>3</sub> alone resulted in only a moderate change in taste-mediated behaviors in contrast to the profound deficit seen in P2X<sub>2</sub>/P2X<sub>3</sub><sup>Dbl<sup>-/-</sup></sup> (KO) animals. This suggests that neither homomeric P2X<sub>2</sub> nor homomeric P2X<sub>3</sub> receptors suffice for normal function in this system.

The near-total loss of neural and behavioral responses to taste stimuli suggests a peripheral origin to the defect. We compared the morphology and innervation of the lingual taste buds in the P2X<sub>2</sub>/P2X<sub>3</sub><sup>Dbl<sup>-/-</sup></sup> (KO) mice with P2X<sub>2</sub>/P2X<sub>3</sub><sup>Dbl<sup>+/+</sup></sup> (WT) animals. As in P2X<sub>2</sub>/P2X<sub>3</sub><sup>Dbl<sup>-/-</sup></sup> (WT) mice, the P2X<sub>2</sub>/P2X<sub>3</sub><sup>Dbl<sup>-/-</sup></sup> (KO) mice showed a
normal complement of taste cells in fungiform, foliate, and circumvallate papillae (Fig. 3, A to D). Cells within the taste buds express the T1R taste receptors as assessed by in situ hybridization (Fig. 3, A to D for T1R1 and T1R2) and display roughly normal proportions of cells expressing gustducin, phospholipase C–β2 (PLC–β2), or serotonin, all markers of taste cells in normal mice. Thus, the peripheral taste apparatus appears intact both structurally and molecularly.

Despite the lack of neural response to any applied tastant in the P2X₃/P2X₃-Dbl/−/− (KO) mice, the animals do exhibit near-normal avoidance to caffeine (bitter to humans) (Fig. 2G) and citric acid (sour) (Fig. 2H). These clear behavioral responses are unexpected, given the total lack of chorda tympani and glossopharyngeal gustatory nerve response to these same substances. Two possibilities may explain the behavioral results. Either these substances are being detected by chemoreceptors that are not taste buds, including those of the larynx, pharynx, esophagus, or gut, or nonlingual taste buds use different neurotransmitters than do lingual taste buds.

The chemical profile (acids and some bitter-tasting substances) of compounds detected by P2X₃/P2X₃-Dbl/−/− (KO) mice is similar to the response profile of laryngeal chemoreceptors innervated by the superior laryngeal nerve (11, 12). The larynx is replete with both taste buds and solitary chemoreceptor cells (SCCs) (13) lying within a specific laryngeal sensory epithelium (14-16). To determine whether or both of these laryngeal chemoreceptors rely on purinergic neurotransmission, we used immunocytochemistry to localize P2X₄ and P2X₇ receptor subunits in transgenic mice in which green fluorescent protein (GFP) is expressed in gustducin-expressing taste cells and SCCs. Laryngeal taste buds were clearly innervated by nerve fibers immunoreactive for P2X₄ (Fig. 3F, red) and P2X₇ receptor subunits. In contrast, laryngeal SCCs, identified by gustducin-driven GFP expression, were not innervated by P2X₄ (Fig. 3G, compare Fig. 3E) or P2X₇ subunit–expressing nerve fibers. These results suggest that laryngeal taste buds may utilize purinergic neurotransmission, whereas laryngeal SCCs do not.

In order to assess whether avoidance of sour and bitter tastants in P2X₃/P2X₃-Dbl/−/− (KO) mice is mediated by the gustatory system, we relied on tantant-induced expression of c-Fos within the brainstem. Activation of the gustatory system by strong tastants quickly activates expression of immediate early genes such as c-fos within the primary taste nucleus (NTS; nucleus of the solitary tract) in the brainstem (15, 16). The lingual gustatory nerves terminate within the rostral and intermediate parts of the NTS, so presentation of tastants to the oral cavity evokes prominent c-Fos expression within the rostral and/or intermediate region of the NTS in both wild-type mice and rats. In contrast, in P2X₃/P2X₃-Dbl/−/− (KO) mice, quinine (but not water) evokes little c-Fos activation in rostral or intermediate portions of NTS, but does evoke significant c-Fos expression in more caudal portions of the nucleus, where the superior laryngeal nerve and general visceral branches of the vagus nerve terminate (Fig. 3, H and I). These findings suggest that the behavioral avoidance seen in P2X₃/P2X₃-Dbl/−/− (KO) mice is mediated by the superior laryngeal nerve or general visceral branch of the vagus conveying signals to the caudal NTS.

Finally, to test for tantant-evoked release of ATP, we used a standard luciferin-luciferase bioluminescence assay to detect ATP release from stripped epithelial preparations. For these experiments, taste bud–bearing epithelia of C57BL/6J mice were stripped from the vallate and foliate taste fields, as well as from nongustatory epithelium devoid of taste buds. Stimulation of the apical surface with buffer resulted in a basal level of ATP release as measured by the luminometer. When a mixture of two bitter compounds was applied to the apical surface of taste bud–bearing epithelium, the luminous flux was significantly greater, which indicated an increase in ATP concent-

![Fig. 2.](image-url)
Our results strongly suggest that ATP serves as a key neurotransmitter linking taste buds to sensory nerve fibers. Criteria for a neurotransmitter include release, the presence of specific receptors, and a mechanism for clearance. Our luminometer data demonstrate taste-stimulated release of ATP. The immunocytochemical studies by Bo et al. (5), as well as our own results, show the presence of postsynaptic P2X receptors, whereas the physiological and behavioral studies demonstrate that the P2X receptors are necessary for

oration (Fig. 4). In contrast, stimulation of non-gustatory epithelium with the bitter mixture evoked little or no ATP release. These findings demonstrate that ATP is released from taste epithelium when it is exposed to appropriate taste stimuli.

Fig. 3. Taste buds in P2X<sub>2</sub>/P2X<sub>3</sub><sup>−/−</sup> (KO) mice (A and C) are normal in terms of cell morphology and histochemistry compared with wild-type mice (B and D). (A and B) Taste buds in circumvallate papillae showing expression of gustducin (green) and T1R2 (red). (C and D) Palatal taste buds showing expression of gustducin (green) and T1R1 (red). (E) Laryngeal solitary chemoreceptor cells are densely innervated in wild-type mice. Gustducin immunoactivity (green) in a solitary chemoreceptor cell showing dense innervation as revealed by immunoreactivity with the pan-neuronal marker PCP9.5 (red). (F) Laryngeal taste buds are innervated by purinceptive nerve fibers expressing P2X<sub>2</sub> (red). Gustducin-expressing taste cells are green. This suggests that laryngeal taste buds, like lingual taste buds, rely on ATP as a transmitter. (G) Laryngeal solitary chemoreceptor cells (green) in a WT mouse are not innervated by P2X-expressing nerve fibers (red), although such fibers do innervate nearby epithelium. This indicates that nerve fibers that innervate laryngeal SCCs utilize a different neurotransmitter and/or receptor system. Compare this image with (E). (H and I) c-Fos immunoreactivity in the laryngeal portion of the nucleus of the solitary tract in P2X<sub>2</sub>/P2X<sub>3</sub><sup>−/−</sup> (KO) mice. Activation of c-Fos in numerous cells (dark spots indicated by red circles) of this area indicates that quinine stimulates the laryngeal nerve, which sends information to this caudal portion of the nucleus. AP, area postrema; NTS, nucleus of the solitary tract; nXII, hypoglossal nucleus; v, 4th ventricle.
Fig. 4. Release of ATP from taste epithelium when stimulated with a bitter mixture containing denatonium and quinine. Nongustatory epithelium (non-TB) and taste bud–bearing epithelial sheets containing either circumvallate (CV) or foliate papillae were placed in an Ussing-type chamber that permits selective application of taste stimuli to the apical membrane. ATP released from the basolateral compartment was collected in the luciferase assay buffer and transferred to the luminometer for measurement of relative light units, which were converted into ATP concentration. Stimulation of taste epithelia with the bitter mixture significantly increases ATP release (mean ± SEM) from CV and foliate tissues relative to non-TB tissue (P < 0.05, t test).

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5. X. Bo et al., Neuroreport 10, 1107 (1999).
10. Materials and methods and supplementary figures are available as supporting material on Science Online.
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Protein p1 is a homolog of the human protein USP9x (an X-linked ubiquitin specific protease, Genbank accession number NP004643) (5, 6) and a mouse protein, FAM (fat facet in mouse, accession number P70398) (7), which share ~98% amino acid identity with one another. FAM and USP9x are homologs of the Dro sophila protein faf (fat facet), which is required for cellularization in early embryos and for cell-fate determination in the Drosophila eye (8). Little is known about the function of USP9x, but both fad and FAM function as deubiquitinating (Dub) enzymes and can regulate protein trafficking (9–14). We refer to the Xenopus protein as xFAM and the human USP9x as hFAM. The proteins correspond to p4, p5, and p7 were identified as the p97 adenosine triphosphatase associated with various cellular activities (AAA ATPase), the nuclear protein localization 4 (Npl4), and the ubiquitin fusion degradation 1 (Ufd1), respectively. The p97 protein forms a homohexameric ring that interacts with the Npl4-Ufd1 heterodimer. The resulting complex functions as a ubiquitin-selective chaperone to regulate protein ubiquitination and degradation (15, 16).

Because all four proteins that coimmuno-precipitated with Survivin are involved in the ubiquitin-mediated signaling, we reasoned that....