The Taste of Carbonation

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Carbonated beverages are commonly available and immensely popular, but little is known about the cellular and molecular mechanisms underlying the perception of carbonation in the mouth. In mammals, carbonation elicits both somatosensory and chemosensory responses, including activation of taste neurons. We have identified the cellular and molecular substrates for the taste of carbonation. By targeted genetic ablation and the silencing of synapses in defined populations of taste receptor cells, we demonstrated that the sour-sensing cells act as the taste sensors for carbonation, and showed that carbonic anhydrase 4, a glycosylphosphatidylinositol-anchored enzyme, functions as the principal CO_2 taste sensor. Together, these studies reveal the basis of the taste of carbonation as well as the contribution of taste cells in the orosensory response to CO_2.

Humans perceive five qualitatively distinct taste qualities: bitter, sweet, salty, sour, and umami (a savory sensation characterized by the taste of monosodium glutamate). Sweet and umami are sensed by members of the T1R family of heterotrimeric guanine nucleotide binding protein (G protein)–coupled receptors (GPCRs) (*1*–*3*); bitter stimuli are detected by T2R GPCRs (*4*–*7*); and sourness is sensed by cells expressing the ion channel PKD2L1 (*8*–*10*). In the tongue, these receptors function in distinct classes of taste cells, each tuned to a specific modality (*7*, *8*, *11*, *12*).

In addition to these well-known stimuli, the taste system appears to be responsive to CO_2 (*13*–*15*). Mammals have multiple sensory systems that respond to CO_2, including nociception (*16*, *17*), olfaction (*18*), and chemoreception essential for respiratory regulation (*19*), yet the molecular mechanisms for CO_2 reception remain poorly defined. Thus, we wondered how taste receptor cells (TRCs) detect and respond to carbonation.

We studied the electrophysiological responses of TRCs to CO_2 by recording tastant-induced action potentials from one of the major nerves innervating TRCs of the tongue [chorda tympani (*15*)]; this physiological assay monitors the activity of the gustatory system at the periphery and provides a reliable measure of TRC function (*12*, *20*). Indeed, the taste system displayed robust, dose-dependent, and saturable responses to CO_2 stimulation. The responses were evident for carbonated drinks (e.g., club soda), CO_2 dissolved in buffer, and even direct stimulation of the tongue with gaseous CO_2 (Fig. 1). In contrast, stimulation with pressured air did not elicit any gustatory response (Fig. 1).

To define the identity of the TRCs needed to taste carbonation, we examined CO_2 responses from engineered mice in which specific populations of TRCs were genetically ablated by targeted expression of attenuated diphtheria toxin [e.g., sweetless, umamiless, sourless mice, etc. (*8*, *21*)] and determined whether their taste systems remained responsive to CO_2. Selective ablation of sour sensing (i.e., PKD2L1-expressing) cells not only abolished all gustatory responses to acidic stimuli, but also eliminated responses to gaseous or dissolved CO_2 (Fig. 1 and fig. S1). These results show that PKD2L1-expressing cells are essential for CO_2 detection.

To identify a candidate CO_2 receptor, we carried out gene expression profiling of sour cells. We reasoned that transcripts for genes involved in carbonation sensing should be enriched in PKD2L1-expressing cells, but that such transcripts would be relatively rare in taste tissue in which PKD2L1 cells have been ablated. Thus, we conducted complementary microarray experiments using mRNA isolated from hand-picked green fluorescent protein (GFP)–labeled sour TRCs, and as a counterscreen, with mRNA from taste buds of animals lacking sour-sensing cells [PKD2L1-DTA mice (*8*)]. One gene, *Car4*, was particularly attractive: It was highly specific for PKD2L1-expressing cells versus other TRC types

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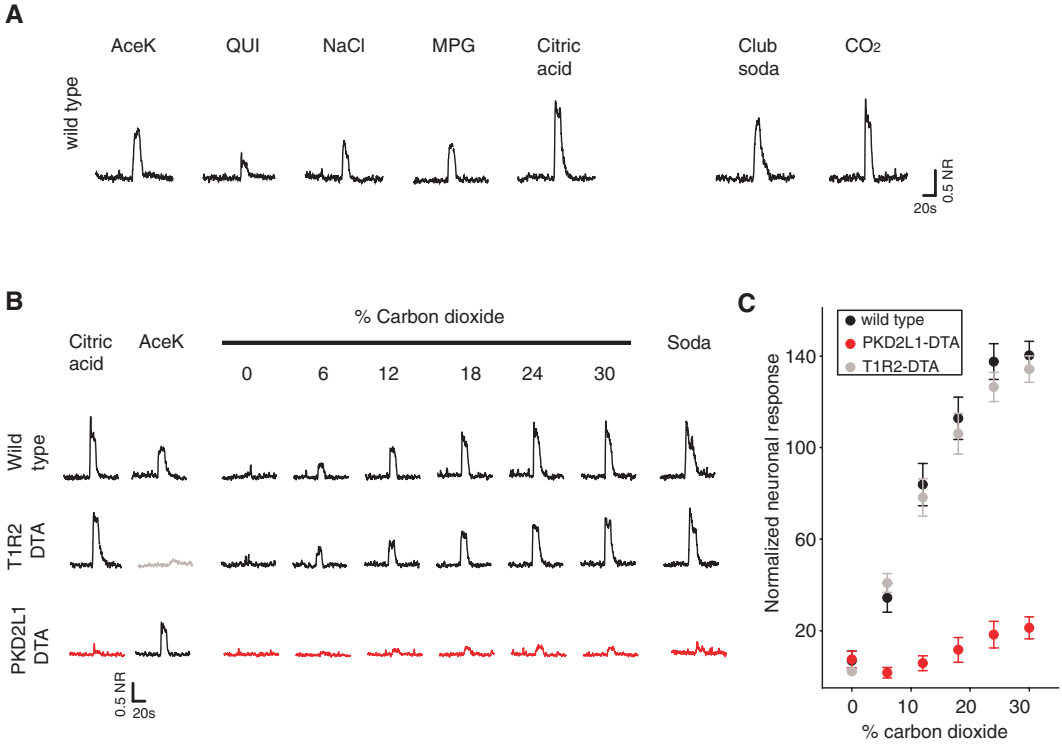

Fig. 1. PKD2L1-expressing sour-sensing cells mediate taste responses to carbonation. (**A**) Wild-type mice show neural responses to carbonated solutions and carbon dioxide. Shown are chorda tympani nerve responses to control sweet (30 mM acesulfame K, AceK), bitter (10 mM quinine, QUI), salty (120 mM NaCl), amino acid (30 mM monopotassium glutamate + 0.5 mM inosine monophosphate, MPG), and sour (50 mM citric acid) stimuli as well as carbonated water (club soda) and gaseous CO_2 normalized to the responses to 250 mM NaCl (NR; see supporting online text). (**B** and **C**) Dose response to CO_2 in wild-type mice or in animals lacking sour-sensing cells (PKD2L1-DTA) and in control animals lacking sweet-sensing cells (T1R2-DTA). (C) Quantitation of carbon dioxide responses in wild-type (n = 6), T1R2-DTA (n = 4), and PKD2L1-DTA (n = 5) animals. Values are means ± SEM of normalized chorda tympani responses.

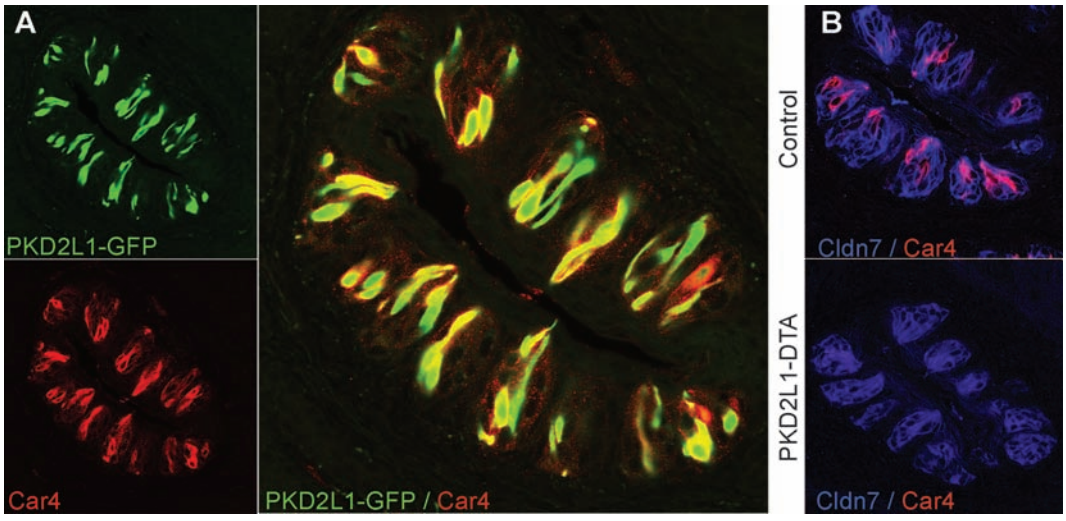

Fig. 2. Selective localization of carbonic anhydrase 4 to PKD2L1-expressing sour cells. (**A**) Immunohistochemical staining of Car4 expression (lower panel, red) in taste buds of transgenic mice in which sour-sensing cells were marked by GFP fluorescence (PKD2L1-GFP; upper panel, green); large panel shows the superimposed double labeling. (**B**) Diphtheria toxin–mediated ablation of sour cells. Upper panel: Double-label immunofluorescence with a marker of TRCs, claudin 7 (Cldn7, blue), and antibodies to Car4 (red). Lower panel: Labeling in PKD2L1-DTA mice stained as above. Shown are sections of foliate papillae; equivalent results were obtained in taste buds from other regions of the oral cavity (fig. S4).

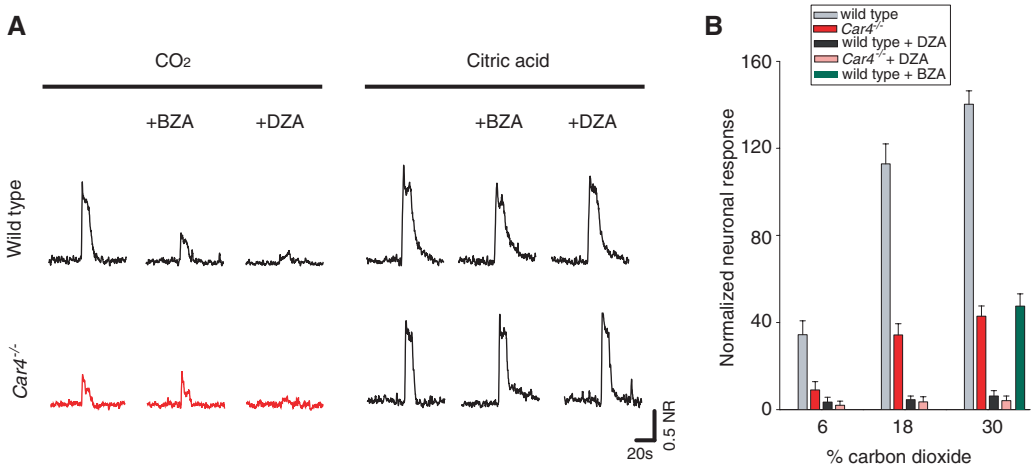

Fig. 3. Requirement of carbonic anhydrase 4 for taste responses to carbon dioxide. (**A**) Representative integrated chorda tympani responses to CO_2 and sour stimulation in wild-type or *Car4*$^{-/-}$ animals exposed to the cell-impermeant carbonic anhydrase inhibitor benzolamide (BZA) or the cell-permeant, broad-spectrum inhibitor dorzolamide (DZA). (**B**) Quantitation of carbon dioxide responses in wild-type and *Car4*$^{-/-}$ animals; means ± SEM (n = 6). Green bar denotes wild-type responses to 30% CO_2 in the presence of BZA. See fig. S5 for responses to other taste stimuli.

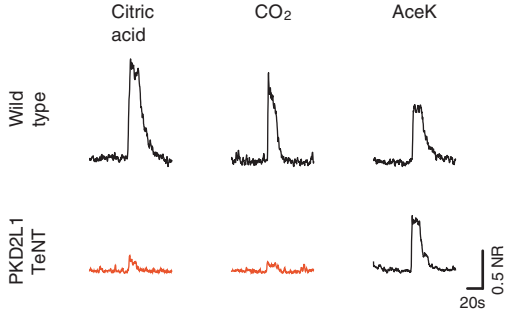

Fig. 4. Requirement of PKD2L1-sour cells for the taste of carbonation. Representative integrated chorda tympani responses to sour, sweet, and CO_2 stimuli in wild-type mice or in animals expressing TeNT in PKD2L1 sour-sensing TRCs. See fig. S5 for responses to additional tastants and quantitative analysis.

(Fig. 2), and moreover it encodes carbonic anhydrase 4, a member of a large family of enzymes implicated in sensing, acting on, and responding to CO_2 in various systems, including chemosensation (*17*–*19*, *22*–*28*).

Carbonic anhydrases (CAs) reversibly catalyze the conversion of CO_2 into bicarbonate ions and free protons (*29*, *30*). Car4 is a mammalian carbonic anhydrase that functions as an extracellular, glycosylphosphatidylinositol (GPI)–anchored enzyme (*30*, *31*). If Car4 is the CO_2 sensor in the taste system, then (i) pharmacological block of extracellular carbonic anhydrases should abolish CO_2 taste responses, and (ii) a knockout of Car4 should selectively affect CO_2 taste detection. We examined nerve responses in the presence of benzolamide, a membrane impermeant inhibitor of carbonic anhydrase (*32*, *33*) (see fig. S2). As predicted, gustatory (chorda tympani nerve) responses to CO_2 were highly susceptible to carbonic anhydrase inhibition (Fig. 3). Next, we characterized *Car4*$^{-/-}$ mutant mice (*32*). Gustatory responses to CO_2 were indeed severely reduced in the mutants at all concentrations tested, whereas responses to other taste stimuli, including sour, were unaltered. Thus, Car4 functions selectively as the main CO_2 sensor in the taste system.

Given that CO_2 taste sensing is completely eliminated in the absence of PKD2L1-expressing cells, we wondered why there are residual taste responses to CO_2 in the *Car4*$^{-/-}$ animals. We hypothesized that the activity of additional carbonic anhydrases in these cells might provide the remaining activity. Indeed, dorzolamide, a broadly acting, membrane-permeable CA blocker (*33*) (see fig. S2) abolished the residual gustatory responses to CO_2, even at the highest CO_2 concentrations tested (Fig. 3). Together, these studies strongly substantiate carbonic anhydrase as the CO_2 receptor, and support a mechanism in which the products of Car4 activity at the extracellular surface of TRCs (i.e., HCO_3^- and H^+) are the principal salient stimuli for detection of carbonation.

How does CO_2 activate the taste system? Bicarbonate does not stimulate TRCs (fig. S3); thus pointing to protons as the relevant signal. Each of

the basic taste modalities is mediated by distinct TRCs, with taste at the periphery proposed to be encoded via labeled lines [i.e., a sweet line, a sour line, a bitter line, etc. (*21*)]. Given that Car4 is specifically tethered to the surface of sour-sensing cells, and thus ideally poised to provide a highly localized acid signal to the sour TRCs, we reasoned that carbonation might be sensed through activation of the sour-labeled line. A prediction of this postulate is that prevention of sour cell activation should eliminate CO_2 detection, even in the presence of wild-type Car4 function. To test this hypothesis, we engineered animals in which the activation of nerve fibers innervating sour-sensing cells was blocked by preventing neurotransmitter release from the PKD2L1-expressing TRCs. In essence, we transgenically targeted expression of tetanus toxin light chain [TeNT, an endopeptidase that removes an essential component of the synaptic machinery (*34*–*36*)] to sour-sensing TRCs, and then monitored the physiological responses of these mice to sweet, sour, bitter, salty, umami and CO_2 stimulation. As predicted, taste responses to sour stimuli were selectively and completely abolished, whereas responses to sweet, bitter, salty and umami tastants remained unaltered (Fig. 4 and fig. S5). However, these animals also displayed a complete loss of taste responses to CO_2 even though they still expressed Car4 on the surface of PKD2L1 cells. Together, these results implicate the extracellular generation of protons, rather than intracellular acidification (*15*), as the primary signal that mediates the taste of CO_2, and demonstrate that sour cells not only provide the membrane anchor for Car4 but also serve as the cellular sensors for carbonation.

Why do animals need CO_2 sensing? CO_2 detection could have evolved as a mechanism to recognize CO_2-producing sources (*18*, *37*)—for instance, to avoid fermenting foods. This view would be consistent with the recent discovery of a specialized CO_2 taste detection in insects where it mediates robust innate taste behaviors (*38*). Alternatively, Car4 may be important to maintain the pH balance within taste buds, and might gratuitously function as a detector for carbonation only as an accidental consequence. Although CO_2 activates the sour-sensing cells, it does not simply taste sour to humans. CO_2 (like acid) acts not only on the taste system but also in other orosensory pathways, including robust stimulation of the somatosensory system (*17*, *22*); thus, the final percept of carbonation is likely to be a combination of multiple sensory inputs. Nonetheless, the "fizz" and "tingle" of heavily carbonated water is often likened to mild acid stimulation of the tongue, and in some cultures seltzer is even named for its salient sour taste (e.g., saurer Sprudel or Sauerwasser).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/326/5951/443/DC1
Materials and Methods
Figs. S1 to S5
References

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Sequential Processing of Lexical, Grammatical, and Phonological Information Within Broca's Area

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Words, grammar, and phonology are linguistically distinct, yet their neural substrates are difficult to distinguish in macroscopic brain regions. We investigated whether they can be separated in time and space at the circuit level using intracranial electrophysiology (ICE), namely by recording local field potentials from populations of neurons using electrodes implanted in language-related brain regions while people read words verbatim or grammatically inflected them (present/past or singular/plural). Neighboring probes within Broca's area revealed distinct neuronal activity for lexical (~200 milliseconds), grammatical (~320 milliseconds), and phonological (~450 milliseconds) processing, identically for nouns and verbs, in a region activated in the same patients and task in functional magnetic resonance imaging. This suggests that a linguistic processing sequence predicted on computational grounds is implemented in the brain in fine-grained spatiotemporally patterned activity.

Within cognitive neuroscience, language is understood far less well than sensation, memory, or motor control, because language has no animal homologs, and methods appropriate to humans [functional magnetic resonance imaging (fMRI), studies of brain-damaged patients, and scalp-recorded potentials] are far coarser in space or time than the underlying causal events in neural circuitry. Moreover, language involves several kinds of abstract information (lexical, grammatical, and phonological) that are difficult to manipulate independently. This has left a gap in understanding between the computational structure of language suggested by linguistics and the neural circuitry that implements language processing. We narrow this gap using a technique with high spatial, temporal, and physiological resolution and a task that distinguishes three components of linguistic computation.

According to linguistic analyses, the ability to identify words, combine them grammatically, and articulate their sounds involves several kinds of

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