Carbonic Anhydrase

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Carbonic Anhydrase IV Mediates the Fizz of Carbonated Beverages

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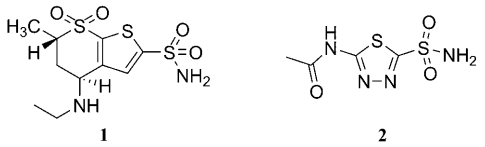
carbonic acid · carbonic anhydrase · metalloenzymes · receptors · taste

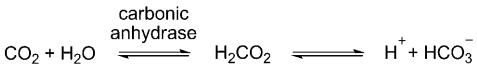
Humans perceive their environment through various sensory systems. While most of our senses including sight, hearing, touch, thermoception, and proprioception are tuned to the detection of physical events, the perception of smell and taste is induced by the activation of chemoreceptor cells in the nose and the oral cavity by volatile and nonvolatile molecules (tastants). Taste comprises five basic taste modalities, namely, bitter, sweet, sour, salty, and umami (savoriness), and is mediated by the activation of taste receptor cells, which are assembled into taste buds distributed across different papillae on the tongue and palate epithelium. In the last decade, numerous genetic and functional studies revealed G-protein-coupled seven-transmembrane proteins as the chemoreceptors responding to sweet (hT1R2/hT1R3),[1,2] bitter (hT2Rs),[3] and umami (hT1R1/hT1R3)[2,4] stimuli, whereas the epithelial sodium channel (ENaC)[5] and the transient receptor potential (TRP) ion channels PKD2L1 and PKD1L3[6] were suggested as receptor proteins for salty and sour taste, respectively. In addition, other types of oral sensations including cooling, pungency, tingling, and astringency seem to be mediated independent of any specific receptor cell through free afferent nerve endings of neurons of the trigeminal nerve in the oral cavity.

Besides taste and trigeminal stimuli, our oral cavity seems to be sensitive for CO_2, the substance responsible for the fizzy and tingling sensation we experience upon consumption of carbonated beverages. This tingling sensation was long linked to the activation of mechanoreceptors in response to bursting bubbles of carbon dioxide.[7] However, experiments under different atmospheric pressure conditions did not lead to a difference in response to CO_2, which would have supported this assumption. Ingestion of carbonated water under hyperbaric conditions, in which bubble formation is prevented, resulted in the same oral sensation as that under normal atmospheric conditions.[8] Moreover a burning, tingling, and slightly numbing orosensation was reported to be perceived even long after the carbonated water had been consumed, thus supporting a chemogenic transduction mechanism.[9]

In psychophysical studies involving lingual application of carbonated water after the tongue had been treated with carbonic anhydrase inhibitors such as dorzolamide or acetazolamide (Scheme 1), drugs typically used to treat glaucoma, epileptic seizures, and altitude sickness, the oral sensation of carbonation was attenuated significantly.[10] Carbonic anhydrases belong to a family of zinc metalloenzymes that reversibly catalyze the conversion of carbon dioxide into hydrogen carbonate and free protons (Scheme 2). These enzymes are of crucial importance to maintaining the acid/base balance in the blood and other tissues as well as transporting carbon dioxide out of tissues.[11]



Scheme 1. Carbonic anhydrase inhibitors dorzolamide (**1**) and acetazolamide (**2**).



Scheme 2. Carbonic anhydrase catalyzed conversion of carbon dioxide and water to give hydrogen carbonate and free protons.

Although a carbonic anhydrase was suggested to be involved in the chemosensory response to CO_2,[11,12] no conclusive molecular mechanism behind "fizzy taste" of carbonated beverages like beer, champagne, and soft drinks had been proposed until a very recent study conducted by Zuker and co-workers.[13] They observed dose-dependent responses to gaseous CO_2, CO_2 dissolved in buffer, and carbonated drinks when they recorded action potentials of major nerves of taste receptor cells (TRCs), whereas stimulation with pressurized air yielded no reaction.

To narrow down the types of TRCs essential to sensing carbonation, transgenic mice were generated in which specific

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populations of TRCs were genetically ablated by targeted expression of attenuated diphtheria toxin. These mice, which could not taste sweet, salty, umami etc., were analyzed for their remaining responsiveness to CO_2. The ablation of sour-sensing cells expressing the proposed sour receptor protein PKD2L1 eliminated the gustatory response to acidic stimuli and surprisingly also the response to CO_2. Subsequent gene-expression profiling of sour-sensing cells in comparison to mRNA from taste buds of animals lacking sour-sensing cells resulted in the identification of the gene *Car4*, which was found to be highly specific for PKD2L1-expressing cells (Figure 1) and to encode carbonic anhydrase IV (CA4). To

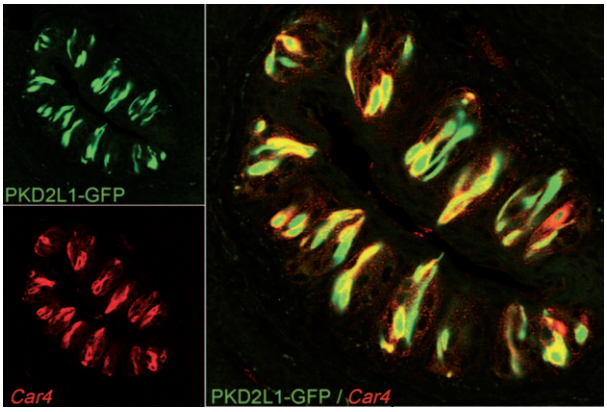

Figure 1. Immunohistochemical staining of *Car4* expression (lower panel, red) in the taste buds of transgenic mice in which sour-sensing cells were marked by GFP fluorescence (PKD2L1-GFP; upper panel, green); large panel shows the superimposed double labeling. Adapted from reference [13]. Reprinted with permission from AAAS.

confirm the role of CA4, a well-known glycosylphosphatidylinositol-anchored membrane enzyme with a molecular weight of 35 kDa (Figure 2),[14] as a selective CO_2 sensor in the taste system, *Car4*-knockout mice were analyzed for their gustatory responses to CO_2 and other taste stimuli. Basic tastants including organic acids were still detected by the knockout mice, whereas CO_2 sensing was strongly reduced even when high levels of 30% CO_2 were applied (Figure 3). Furthermore, blocking of the carbonic anhydrases by the inhibitor dorzolamide abolished quantitatively gustatory responses to CO_2, giving strong evidence for CA4 as the primary carbon dioxide detector.[13] In addition, engineered animals, in which the activation of nerve fibers innervating sour-sensing cells was blocked by preventing neurotransmitter release from PKD2L1-expressing TRCs, did not respond to either sour taste stimuli or to CO_2. Since these animals were still responsive to the other basic taste modalities, sour cells were proved to be the cellular sensors for carbonation. As CO_2 was found to act not only on the sour taste system but also on other somatosensory pathways,[10] the fizzy and tingling perception of carbonated beverages is likely to be due to a multimodal sensation comprising gustatory as well as somatosensory inputs. It is interesting to note that these findings explain the phenomenon of the so-called "champagne blues", which was reported already 20 years ago.[15]

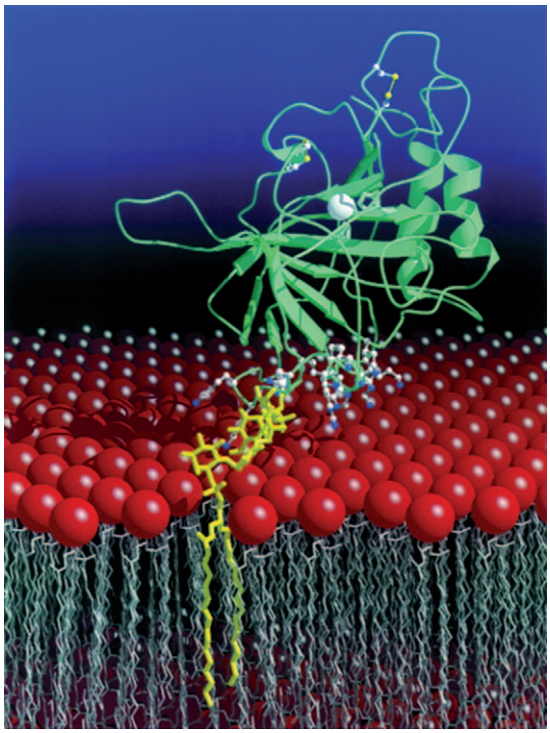

Figure 2. Representation of the carbonic anhydrase IV anchored to a membrane by a glycosylphosphatidylinositol tail (yellow); the active site zinc ion of the enzyme appears as a white sphere. Adapted from reference [14c]. Copyright (1996) National Academy of Sciences, USA.

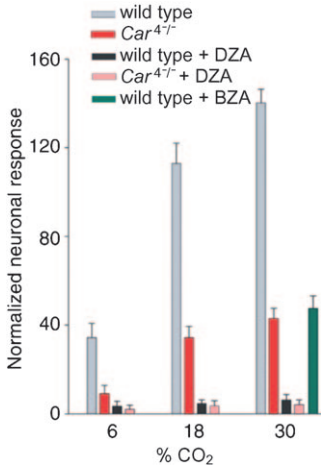

Figure 3. Carbon dioxide responses of wild-type (gray) and *Car4*-knockout (red) animals, after exposure to the membrane-permeable CA blocker dorzolamide (DZA), or the cell-permeable carbonic anhydrase inhibitor benzolamide (BZA). Adapted from reference [13]. Reprinted with permission from AAAS.

Mountain climbers who had taken the carbonic anhydrase inhibitor acetazolamide (Scheme 1) to prevent altitude sickness reported that the champagne or beer consumed to celebrate the triumph at the peak tasted "like dishwater".[15] This clearly demonstrates that the fizzy sensation perceived by humans upon consumption of carbonated beverages is mediated by carbonic anhydrase IV.

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