Supporting Online Material for

The Taste of Carbonation

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Published 16 October 2009, Science 326, 443 (2009)
DOI: 10.1126/science.1174601

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Materials and Methods

Mouse strains and immunostaining

Car4<sup>−/−</sup>-mice (1) and Rosa26-flox-STOP-TeNT (2) were as described; PKD2L1-TeNT mice (Figure 4) expressed TeNT in more than 90% of PKD2L1 cells (data not shown). All other mice including double positive Cre-driver/reporter lines PKD2L1-DTA (sour-less), PKD2L1-GFP and T1R2-DTA (sweet-less) animals were as described and characterized before (3). We also used a DTA-based strategy to generate mice lacking all T1R and T2R-expressing taste receptor cells (T1R3-ires-Cre, defining progenitors of sweet, umami and bitter TRCs); these animals exhibited a compound loss of sweet, bitter and umami taste but showed no deficits in either sour taste or the taste of carbonation (Supplementary Fig. S1).

Antibodies to PKD2L1 (3) and Car4 (4) were described previously, goat anti-claudin7 was from Santa Cruz; immunocytochemistry used standard techniques (3, 5). Images were obtained using a Leica SP2 TSC confocal microscope; 1-2 μm optical sections were recorded to ensure that any overlapping signal originated from single cells.

Identification of Car4 as a candidate CO<sub>2</sub> receptor

We used a strategy that combined bioinformatics, differential gene array screening, and in situ hybridizations to identify candidate CO<sub>2</sub> receptors. Taste buds from control mice, from GFP-labeled TRCs, and from mice lacking sour cells (PKD2L1-DTA animals) were used to prepare cDNA for hybridization to Affymetric Mouse Gene 1.0 ST microarrays.
Bioinformatic analysis of genes containing at least 1 transmembrane domain (Gene Ontology Cellular Component 0016020) identified Car4 as the most dramatically underexpressed gene in the sample derived from PKD2L1-DTA taste tissue (>20 fold reduced signal relative to wild type). Examination of expression of other membrane-bound carbonic anhydrases (Car9 and Car14) showed no significant expression in PKD2L1-expressing cells.

**Nerve Recordings**

Lingual stimulation and recording procedures were performed as previously described (6, 7). All data analyses used the integrated response over a 15 s period immediately after the application of the stimulus. Tastants used for nerve recordings were: 30mM, 60mM acesulfameK (AceK); 30mM mono potassium glutamate + 1mM inosine mono phosphate (Glu); 10mM quinine hydrochloride (Qui); 120mM, 250mM sodium chloride (NaCl); 10mM, 50mM citric acid. Concentrations of carbonic anhydrase inhibitors used were: 100μM Benzolamide (BZA), 0.5% Dorzolamide hydrochloride (TRUSOPT ophthalmic solution, Merck). Carbonated solutions were made using a Soda-Club home soda maker and gaseous CO2 stimulation was done at a constant flow rate by mixing different ratios of air and CO2. The mean response to 250mM NaCl was used to normalize responses to each experimental series in the wild type, PKD2L1-DTA/TeNT, T1R2-DTA and Car4-/- animals.

**Carbonic anhydrase inhibitors in taste cells**
Freshly-peeled taste epithelium (8) was pre-incubated with BZA (100 μM), DZA (0.5%) or control buffer for 6 min. prior to CO2 stimulation. Intracellular pH (pHi) was monitored with SNARF-1 dextran (Invitrogen) as described (9) using a 5-Live confocal microscope (Zeiss). Carbonated water was buffered to pH 7.4 using 72 mM NaHCO3 and applied to the epithelium for 2 min. ΔpHi was calculated as the difference in pHi between the beginning and end of stimulation.
Supplementary Figure Legends

Supplementary Figure S1: Mice in which T1R and T2R cells have been ablated with diphtheria toxin show compound loss of all responses to sweet (AceK, 50 mM acesulfame K), umami (MPG, 30 mM monopotassium glutamate plus 0.5 mM inosine monophosphate) and bitter (CYX, 100 M cycloheximide) tastants but still respond to salty (250 mM NaCl) and sour (50 mM citric acid) stimuli. Importantly, responses to CO2 are unaffected by ablation of the T1R and T2R cells.

Supplementary Figure S2: Permeability of BZA and DZA in taste receptor cells. (a) Carbonated water decreases intracellular pH ($pHi$) in TRCs. $pHi$ changes were monitored as described in Methods in the presence of the carbonic anhydrase inhibitors BZA (100 μM, blue trace), or DZA (0.5%, red trace); the black trace denotes control conditions in the absence of inhibitors. The pH stimulus (carbonated water) was applied during the period indicated by the gray box. A significant decrease in $pHi$ due to the catalytic action of carbonic anhydrases was observed for control buffer or BZA, but not for DZA. (b) Averaged $pHi$ changes from 9 TRCs; the values are mean ± sem.

Supplementary Figure S3: Potassium bicarbonate does not elicit taste responses; for comparison responses to 50 mM citric acid and 100 mM ammonium chloride are shown.

Supplementary Figure S4: Car4 is expressed in subsets of TRCs in all classes of taste buds of the oral cavity. Shown are sections from circumvallate (CV), fungiform (Fungi) and palate taste-buds; Car4 (green) and claudin 7 (Cldn7, red). DTA mediated ablation of
sour cells (PKD2L1-DTA, lower panels) completely eliminates taste bud expression of Car4 throughout the oral cavity.

Supplementary Figure S5: Average neural responses to sour, sweet, bitter amino-acid (umami) and salty taste. Note the specific loss of responses to AceK in the T1R2-DTA animals and the selective loss of citric acid responses in the PKD2L1-DTA and PKD2L1-TeNT animals. The values are mean ± s.e.m (n=4) of chorda tympani responses normalized to the response of 250mM NaCl.
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References


