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The other side of the moon: bitter taste receptors activated by vanillin and capsaicin

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Introduction: vanilla and chilli pepper are widely used in food preparations worldwide for their sensory properties, mainly related to the fragrance (odorant properties) of vanillin and the chemestetic properties of capsaicin respectively. Both vanillin and capsaicin are also known for their capability to elicit bitterness as a secondary sensory sensation (1,2), but which TAS2R receptors are involved has never been reported.

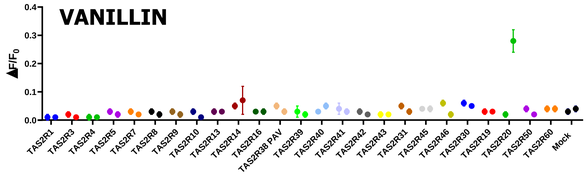
Experimental: all hTAS2Rs have been transfected into a heterologous cell-based assay system and screened for activation. Vanillin and capsaicin were first tested at one concentration based on the maximum concentration tolerated by the assay. Positive receptor-compound combinations were retested in a full concentration-response experiment at eight concentrations. References were dissolved in assay buffer or in a mixture of assay buffer and DMSO, never exceeding final DMSO 0.5% (v/v) to avoid toxic effects and possible artefacts on transfected cells.

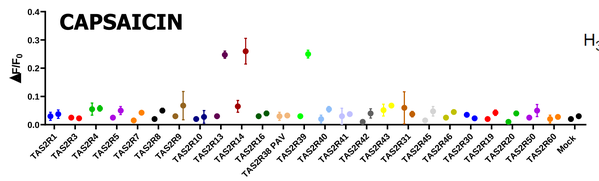
Vectors and cDNAs: all hTAS2R cDNAs were cloned into a pcDNA5/FRT expression vector 3' of the first 45 amino acids of the rat somatostatin receptor 3 (3). The cDNAs for the chimeric G protein alpha subunit was cloned into a pcDNA6 expression vector.

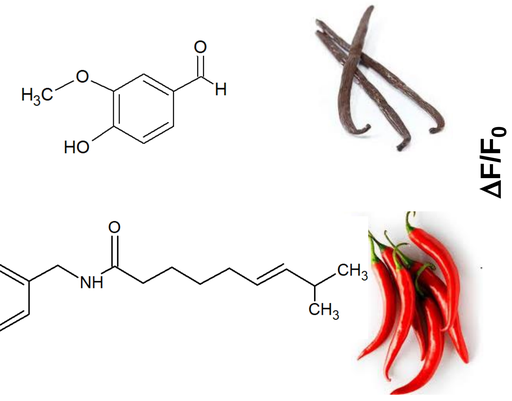
Cell Culture and Transfection: HEK293T cells were cultured in high glucose DMEM with stable L-glutamine, 10% (v/v) FBS and 1% (v/v) Pen/Strep 10K/10K stock. A stable cell line was produced by constitutively expressing a chimeric G protein alpha subunit. The cDNAs for the different TAS2Rs were transiently transfected using Lipofectamine2000. Cells were plated at a density of 20,000 cells/well in 384 plates and analyzed 24 h after transfection.

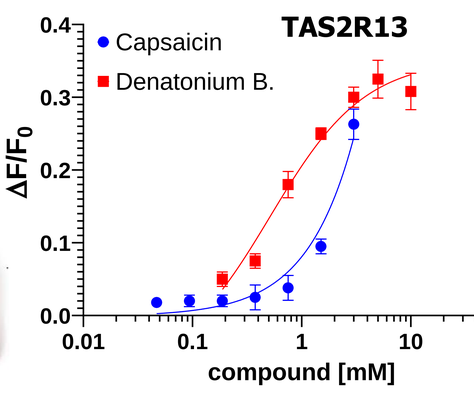
Calcium Imaging Analysis: a solution of Cal520-AM calcium-sensitive dye and probenecid was prepared using the assay buffer and diluting the kit components according to the manufacturer's instructions. 24 h after transfection, cells were loaded with Cal520-AM and probenecid solution and incubated 3 h at room t. After incubation, cells were washed once with the assay buffer before data acquisition.

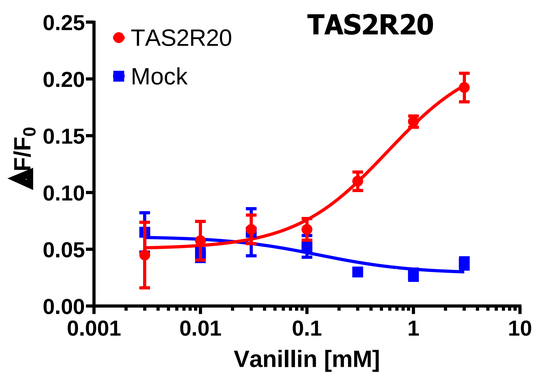
Quantification: cellular calcium traces were recorded at 515 nm following excitation at 490nm by a fluorometric imaging plate reader (FLIPRTETRA, Molecular Devices equipped with ICCD Camera from Stanford Photonics, INC). Calcium kinetics were visualized using Molecular Devices Screenworks 4.1. For the calculation of concentration-response curves, responses were calculated as the difference between maximal and minimal Relative Fluorescence Unit (RFU) values in a selected time window and were normalized to basal well fluorescence (timepoint 1, before compound injection) in order to compensate for differences in cell density (ΔF/F0). All the results are averages of at least four replicates. All calculations and plots were made using Microsoft Excel 365 and GraphPad Prism 8.0.



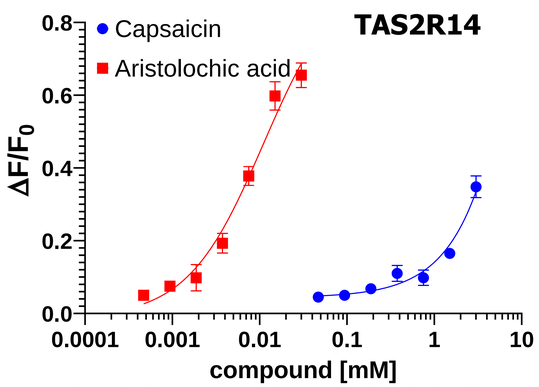


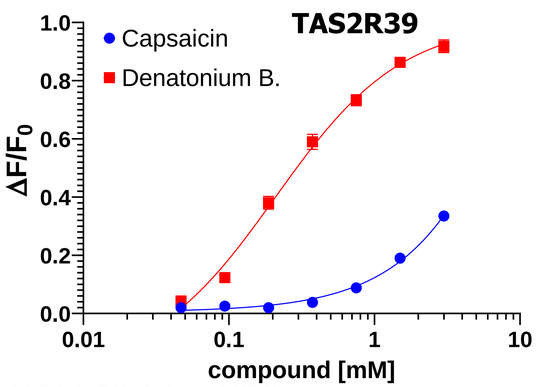






Assay buffer or vanillin were tested at 1 mM on all hTAS2Rs. Only TAS2R20 showed specific activation by vanillin. Activation of TAS2R20 by vanillin was concentration dependend with an EC50-value of 0.57 mM.





Assay buffers or capsaicin were tested at 1 mM on all hTAS2Rs. TAS2R13, TAS2R14 and TAS2R39 showed specific activation, which was concentration dependend, but did not reach saturation. Estimated EC50-values were >2 mM for TAS2R13 and >3 mM for TAS2R14 and TAS2R39.

Conclusions: we identified TAS2R20 to be the principal detector of vanillin as no other TAS2R responded to this compound.

capsaicin activated three TAS2Rs, namely TAS2R13, TAS2R14 and TAS2R39. These outcome could be useful to improve the overall sensory profile of these broadly used food ingredients. Moreover, since TAS2Rs have been proven to have a role in biological functions of physiological and pathological relevance, their activation by such common bioactive compounds could be of interest in sensory nutrition and other health related aspects.

References:
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3. W. Meyerhof, C. Batram, C. Kuhn, A. Brockhoff, E. Chudoba, B. Bufe, G. Appendino, M. Behrens. The molecular receptive ranges of human TAS2R bitter taste receptors. *Chemical Senses* 35 (2010), 157-170.