Bitter Taste Receptors on Airway Smooth Muscle: A Surprising Target for Bronchodilator Therapy

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No Conflicts of Interest
Serendipity

“"The faculty of phenomenon of finding valuable or agreeable things not sought for” – Webster-Merriam

“"to look for a needle in a haystack and coming out with the farmer’s daughter” – Julius H. Comroe M.D.

"a silly fairy tale, called The Three Princes of Serendip: as their highnesses traveled, they were always making discoveries, by accidents and sagacity, of things which they were not in quest of....” – Horace Walpole 1754

Asthma

22.2 Million People Currently Diagnosed with Asthma

13.6 Million Unscheduled Office Visits

1.8 Million Emergency Room Visits

½ Million Hospitalizations

4000 Related Asthma Deaths (11 deaths/day)

National Center for Health Statistics, CDC, 2005; http://www.cdc.gov/nchs/products/pubs/pubd/hestats/asthma/asthma.html
Chronic obstructive pulmonary disease (COPD) characterizes a group of diseases that cause airflow obstruction, including emphysema and chronic bronchitis. In 2008, COPD became the third leading cause of death in the United States.


Obstructive Lung Disease Pathophysiology

- Airway inflammation
- Airway smooth muscle constriction and in the case of asthma, proliferation of smooth muscle
Anticholinergic – ipratropium: 1977
Anti-inflammatory – beclomethasone: 1976
Anti-leukotriene – zafirlukast: 1996
Omalizumab (monoclonal Ab to IgE): 2003
Bitter Taste Receptors (TAS2Rs)

- G-protein coupled receptors (GPCRs) that signal via the G-protein alpha gustducin
- Avoidance of bitter tasting compounds is universal among vertebrates
- Generally accepted to have evolved in taste buds to avoid ingestion of toxic substances
- Twenty Five TAS2Rs have been identified in humans
TAS2R Agonists on Taste Buds Cause Release of Intracellular Calcium and Depolarization

Bronchoconstriction via Gq-receptors

leukotrienes, acetylcholine, histamine

Airway smooth muscle contraction
Airway smooth muscle relaxation

Hypothesis: Bitter Tastants Should Cause Bronchoconstriction

- Bitter taste receptors evoke Ca\(^{2+}\) release
- Signal transduction in airway smooth muscle seemed like that in taste cells (gust, βγ, PLC, Ca\(^{2+}\))
- Like in the tongue, an aversion response could be bronchoconstriction/shortness of breath: leave the environment
- Potential cause of occupational asthma (agents in the environment evoke asthma physiology which is not present at home)
Night Science

not hypothesis driven
risky
chase after some aberrancy that may lead to
something really new
often goes against current dogma
chaotic, unpredictable from day to day
new methods for the lab (or create new methods)
“can do” attitude regardless of technical challenge
not what you write a grant about (at least at first)
data seem to appear after 7 PM

Modified from François Jacob Of Flies, Mice, and Men
Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction

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Real Time PCR of TAS2Rs in Cultured Human Airway Smooth Muscle (HASM) Cells

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ratio ADRB2</th>
</tr>
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<tbody>
<tr>
<td>TAS2R10</td>
<td>3.96 ± 0.393</td>
</tr>
<tr>
<td>TAS2R14</td>
<td>3.51 ± 0.397</td>
</tr>
<tr>
<td>TAS2R31</td>
<td>3.41 ± 0.498</td>
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<tr>
<td>TAS2R35</td>
<td>1.76 ± 1.000</td>
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<tr>
<td>TAS2R4</td>
<td>1.45 ± 0.271</td>
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<tr>
<td>TAS2R19</td>
<td>1.37 ± 0.249</td>
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<tr>
<td>TAS2R3</td>
<td>0.83 ± 0.079</td>
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<tr>
<td>TAS2R20</td>
<td>0.71 ± 0.202</td>
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<tr>
<td>TAS2R45</td>
<td>0.70 ± 0.118</td>
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<tr>
<td>TAS2R5</td>
<td>0.48 ± 0.033</td>
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<tr>
<td>TAS2R30</td>
<td>0.31 ± 0.060</td>
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<tr>
<td>TAS2R8</td>
<td>0.31 ± 0.034</td>
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<tr>
<td>TAS2R13</td>
<td>0.26 ± 0.037</td>
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<tr>
<td>TAS2R42</td>
<td>0.26 ± 0.009</td>
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<tr>
<td>TAS2R46</td>
<td>0.25 ± 0.041</td>
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<tr>
<td>TAS2R1</td>
<td>0.17 ± 0.027</td>
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<tr>
<td>TAS2R8</td>
<td>0.15 ± 0.007</td>
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<tr>
<td>TAS2R39</td>
<td>ND</td>
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<tr>
<td>TAS2R43</td>
<td>ND</td>
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<tr>
<td>TAS2R7</td>
<td>ND</td>
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<tr>
<td>TAS2R40</td>
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<td>TAS2R16</td>
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<tr>
<td>TAS1R1</td>
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<tr>
<td>TAS1R2</td>
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<tr>
<td>ADRB2</td>
<td>1.0 (reference)</td>
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<tr>
<td>ADORA1</td>
<td>2.43 ± 0.446</td>
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<tr>
<td>LTB4R</td>
<td>0.29 ± 0.056</td>
</tr>
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</table>
**Immunofluorescence Confocal Microscopy**

Positive by RT-PCR

Negative by RT-PCR

**Agonist-Mediated cAMP release**
Dose Dependent Stimulation of $[\text{Ca}^{2+}]_i$ in HASM

Accumulation of Phosphoinositides in Response to Bitter Agonists

Bitter taste receptor agonists cause phosphoinositide accumulation to a similar degree as histamine in cultured HASM cells.
Elucidating Mechanism of Bitter Agonist induced \([Ca^{2+}]_i\) in HASM

2APB: Inhibits IP₃ Receptors
Gallein: Inhibits G-protein βγ
U73122: Inhibits Phospholipase C

Unexpected Relaxation of Mouse Trachea by Bitter Tastants
Human Bronchial Rings Studies

TAS2R agonist-mediated Relaxation is Rapid and Fully reversible

Sub-cellular Localization of Bitter Tastant Mediated \([Ca^{2+}]_i\) Signaling in Human Smooth Muscle

2D: 0.22 \(\mu\)m/pixel, 512 pixels/line, 256 lines/image every 0.5 sec
Linescan: 0.075 \(\mu\)m/pixel, 512 pixels/line, 10,000 lines/image every 0.002 sec

TAS2R Agonists cause BKca2+ Dependent Hyperpolarization
Relaxation of Mouse Trachea is BKca2+ Dependent

Iberiotoxin: Inhibits BKca2+

TAS2R signaling in ASM-model
Wide Variety of Bitter Compounds

- Naturally Occuring:
  - Hops
  - Grapefruit Extract
  - Teucrium Pollium (Iranian Herb used to treat asthma)
- Established, FDA approved drugs:
  - Chloramphenicol
  - Famotidine
  - Dextromethorphan
  - Diphenhydramine

Conclusions

- TAS2Rs are on many tissues and probably have many roles that we are just beginning to understand
- From the beginning bitter taste receptors had physiologic responses that were the opposite of what we expected.
- They now provide a new potential target of therapy for bronchodilation.
Acknowledgements

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Kathryn Mihlbachler
Esther Moses

Additivity with β-agonists
Bitter Tastants Relax Isolated Human Airway Smooth Muscle Cells as Assessed by Magnetic Twisting Cytometry

Unaffected by PKA inhibitor (H89)
Blocked by PLC inhibitor (U73112)
Blocked by Ca\(^{2+}\)-dependent K\(^{+}\) channel antagonist (ChTX)
Blocked by the BK\(_{Ca}\) channel antagonist IbTX

Inhaled Quinine Decreases Airway Resistance in Ovalbumin-Sensitized Mouse Model of Asthma
Diverse Bitter Tastants Stimulate $[\text{Ca}^{2+}]_i$ in Human Airway Smooth Muscle Cells

Magnitude Similar to Known Airway Constrictive Agents

TAS2R Agonists Relax Mouse Trachea!

Dose Response Curves:
- Quinine
- Chloroquine
- Denatonium

- CHLORO 3.0 mM
- QUININE 1.0 mM
TAS2R Agonists Relax Human Bronchi *ex vivo*

Depletion of Ca\(^{2+}\) by Thapsigargin

LINK: [Ca\(^{2+}\)], release to relaxation
TAS2R Agonists Hyperpolarize the ASM Membrane

- IbTx sensitive
- Potential "sharing" of the \([\text{Ca}^{2+}]_i\) pool

TAS2Rs undergo rapid agonist-promoted phosphorylation and functional desensitization, potentially via GRK2
HSV-tagged TAS2Rs expressed in HEK293 cells
Show Agonist-promoted Upregulation with Chronic Exposure
Screening Method for TAS2R Agonists

Calcium assay on cultured HASM

Dose response curve with n>3 calcim assay

Dose response curve in mouse trachea

Antibodies to TAS2R10 Block [Ca\(^{2+}\)]_i Stimulation by Strichnine
Sub-cellular Localization of Bitter Tastant-Mediated \([\text{Ca}^{2+}]_i\) Signaling in HASM by High Resolution Fluorescence Imaging