Proton-sensing receptors- therapeutic targets in the management of asthma?

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“Proton-sensing receptors-therapeutic targets in the management of asthma?”
The Speaker Declares No Conflicts of Interest
G protein-coupled receptors pretty much explain all biological phenomena and are the only thing worth studying.

**Biological functions**
- smell and taste (~1000 types of receptors)
- perception of light
- neurotransmission
- function of endocrine and exocrine glands
- chemotaxis
- exocytosis
- control of blood pressure
- embryogenesis
- development
- cell growth and differentiation
- HIV infection
- oncogenesis

Rho GEF
Na\(^+\)-H\(^+\) exchangers
GPCR agonists are physiological, pathological, and therapeutic regulators of ASM contractile state

1. Contractile state is a function of the dynamic balance of (pro-contractile) Gq-coupled vs (pro-relaxant) Gs-coupled GPCR activation.

2. Physiological: Parasympathetic innervation providing acetylcholine (ACh) activating Gq-coupled m3 muscarinic acetylcholine receptor (m3mACHr) is principal regulator of physiological tone.
GPCR agonists are physiological, pathological, and therapeutic regulators of ASM contractile state

3. Pathological: Inflammation can cause increased parasympathetic ACh release (m3mAChR), and numerous inflammatory mediators (e.g. histamine, LTC4/LTD4, endothelin, serotonin) can activate Gq-coupled receptors on ASM.

4. Therapeutic: Many anti-asthma/COPD drug either: 1) block Gq-coupled receptors (monteleukast for CysLT1R, tiotropium for m3mAChR); or 2) activate bronchodilatory Gs-coupled receptors (beta-agonists).
Proton-sensing GPCRs

• Subfamily of GPCRs linked by sequence similarity:
  – OGR1, G2A ? (Gq)
  – GPR4, TDAG8 (Gs)

• Can exhibit high level of constitutive activity

• Originally thought to be receptors activated by lysolipids

• Subsequently found to signal in response to lowering extracellular pH
Sequence alignment identifies OGR1, GPR4, TDAG8, and G2A as family of GPCRs
Expressed OGR1 exhibits constitutive and pH-dependent IP production
pH-dependent accumulation of intracellular cAMP by GPR4, TDAG8
How do protons activate these GPCRs?
Predicted structure, proton-sensing histidine residues in OGR1, GPR4, and TDAG8
Mutation of putative proton-sensing histidines inhibits pH sensing by OGR1
$G_q$-coupled receptor signaling in ASM

- Contraction
  - $G_q$-coupled receptor
  - PLC
  - IP$_3$
  - MLCK
  - MLCC

- Growth
  - PLA2
  - p42/p44
  - CaM
  - eicosanoids
  - COX
  - AA

- DAG
- Ca$^{2+}$

- CoA
- H$_2$N
- COOH

- Alpha (GTP)
- Beta (GTP)
G_s-coupled receptor signaling

Relaxation
Gene Regulation
Channel Regulation
Growth (-)
Gs-coupled receptors antagonize Gq-coupled receptor-mediated contraction

- Primarily via PKA activation
- Inhibits increase in intracellular Ca$^{2+}$
- Inhibits cellular sensitization to Ca$^{2+}$
PKA targets:

A. Controlling Ca\(^{2+}\) release entry:
   1. Gq-GPCR
   2. Gq
   3. PLC
   4. Phospholamban/IP3R
   5. K+ and Ca\(^{2+}\) channels

B. Calcium Sensitization:
   - Calcium/calmodulin/MLCK
   - Rho Kinase reg of MLCP
Objectives

• Characterize the proton sensitive GPCRs in the ASM
• Determine the intracellular signaling mechanisms activated by proton sensitive receptors in the ASM
• Determine the functional consequences of changing pH in the microenvironment of the ASM
Current proposed mechanisms

1. Reflex Mechanism
2. Reflux Mechanism

Sensory Neuron

H⁺ H⁺ H⁺ H⁺ H⁺
SP NKA ACh

Efferent Neuron

1

Trachea-Bronchi

CNS

1 2

Esophagus
Acid Stress and Asthma Pathophysiology

Reflex Mechanisms

Acid-induced cough bronchial hyperreactivity bronchoconstriction eosinophil necrosis mucous plugging

<table>
<thead>
<tr>
<th>Respiratory fluid</th>
<th>Condition</th>
<th>Measured pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBC (deaerated to remove CO2)</td>
<td>Health</td>
<td>7.7±0.49</td>
</tr>
<tr>
<td></td>
<td>Asthma exacerbation</td>
<td>5.2±0.21</td>
</tr>
<tr>
<td></td>
<td>Stable mild asthma</td>
<td>7.6 (7.55-7.65)</td>
</tr>
<tr>
<td></td>
<td>Stable moderate asthma</td>
<td>7.27 (CI, 7.15-7.39)</td>
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<tr>
<td></td>
<td>Stable COPD</td>
<td>7.16 (CI, 7.09-7.23)</td>
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<tr>
<td></td>
<td>COPD exacerbation</td>
<td>6.25</td>
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<tr>
<td></td>
<td>Stable bronchiectasis</td>
<td>7.11 (CI, 7.04-7.19)</td>
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<tr>
<td></td>
<td>Intubated: healthy</td>
<td>7.8±0.28</td>
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<tr>
<td></td>
<td>Intubated: sepsis</td>
<td>5.92</td>
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<tr>
<td></td>
<td>Intubated: ARDS</td>
<td>6</td>
</tr>
<tr>
<td>Tracheal fluid in vivo (transcricoid pH probe)</td>
<td>Health w/GER episodes</td>
<td>7.0 (6.5-7.4)</td>
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<tr>
<td></td>
<td></td>
<td>To &lt;4.0</td>
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<td>Bronchial fluid in vivo (pH probe inserted through bronchoscope)</td>
<td>Health</td>
<td>7.1±0.1</td>
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<td>Airway submucosal gland secretion (explanted tissue)</td>
<td>Health</td>
<td>6.97</td>
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<td>Nasal fluid in vivo (pH probe)</td>
<td>Health</td>
<td>7.4-7.9</td>
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<tr>
<td></td>
<td>Health</td>
<td>6.9-7.4</td>
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<tr>
<td></td>
<td>Health</td>
<td>7.31 (7.2-7.5)</td>
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<tr>
<td></td>
<td>Allergic rhinitis</td>
<td>7.8-8.5</td>
</tr>
<tr>
<td></td>
<td>Chronic hypertrophic rhinitis</td>
<td>7.14 (6.6-7.6)</td>
</tr>
</tbody>
</table>

Airway pH tends to alkaline, but decreases with allergic inflammation

From Ricciardolo, Gaston, and Hunt *JACI* 2004
Corticosteroids reverse the reduced airway pH associated with acute asthma exacerbations
Proton-sensing GPCRs may represent another mechanisms mediating effects of more subtle decreases in airway pH.
↓pHo activates cAMP/PKA, Akt, and p42/p44 in ASM

(A) Bar graph showing cAMP levels at different pH values. 

(B) Western blot analysis showing VASP, p-Akt, p-p42/p44, and β-actin expression at various pH levels and time points.

(C) Western blot analysis showing VASP, p-Akt, p-p42/p44, and β-actin expression with pH values ranging from 8.0 to 6.4, including a control for BK.
↓pH0 activates cAMP/PKA, Akt, and p42/p44 in ASM
PKA activation by ↓pHo not necessarily dependent on COX: Pleiotropic signaling?
Akt, p42/p44 activation by \( \downarrow \text{pH}_0 \) is not via RTK transactivation.
↓pHo stimulates PI hydrolysis, Ca\(^{2+}\) mobilization
Expression of proton sensitive receptors in ASM

PCR of GPCRs in HASM and Airway Epithelium

<table>
<thead>
<tr>
<th></th>
<th>GPR4</th>
<th>OGR1</th>
<th>TDAG8</th>
<th>GPR4</th>
<th>OGR1</th>
<th>TDAG8</th>
<th>GPR4</th>
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<td>HASM</td>
<td>HEK w/ GPR4</td>
<td>HEK w/ GPR4</td>
<td>HEK w/ GPR4</td>
<td>HEK w/ GPR4</td>
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<td>Airway Epithelium</td>
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</tbody>
</table>

Expression of proton sensitive receptors in ASM
OGR1 knockdown reduces PKA, p42/p44 activation by \( \downarrow \text{pH} \)
OGR1 knockdown reduces $\text{Ca}^{2+}$ mobilization by $\downarrow\text{pH}_{\text{o}}$
Signaling looks good.

What about function?
ASM tissue contracts in a pH dose-dependent manner
ASM cell contraction: Magnetic Twisting Cytometry (MTC)
Cells contract, too

![Graph showing cell stiffness over time for different pH values. The graph includes a line chart and a bar chart comparing cell stiffness before and after treatment at various pH levels.]
...and OGR1 knockdown inhibits this contraction

**Figure:**

- **Left Panel:**
  - Title: SCR siRNA pH 6.8
  - Title: OGR1 siRNA pH 6.8
  - Y-axis: Cell Stiffness (ratio of baseline)
  - X-axis: Time (s)

- **Right Panel:**
  - Title: SCR siRNA Histamine
  - Title: OGR1 siRNA Histamine
  - Y-axis: Cell Stiffness (ratio of baseline)
  - X-axis: Time (s)

**Legend:**

- SCR siRNA pH 6.8
- OGR1 siRNA pH 6.8
- SCR siRNA Histamine
- OGR1 siRNA Histamine
...and in the obligatory mouse experiment

Cell Stiffness (ratio of baseline) vs. Time (s)

- OGR1+/+ pH 6.8
- OGR1-/- pH 6.8

pH 8.0→6.8
...and in the obligatory mouse experiment

![Graph](image)

Cell Stiffness (ratio of baseline)

Time (s)

pH 8.0→6.8
Summary of results

• Stimulation of ASM cells with increasing concentration of protons leads to activation of p42/p44 MAPK and calcium elevation suggesting Gq-mediated responses

• Acid stimulation of ASM cells also results in the activation of PKA that is not necessarily COX dependent

• Protease activity cannot account for activation of RTK pathways

• Acid contracts ASM tissue and ASM cells
Summary of results

• OGR1 is the predominant proton sensitive receptor in the ASM cells
• OGR1 knockdown in human ASM inhibits acid-induced PKA and Ca\(^{2+}\) mobilization
• OGR1 knockdown in human ASM inhibits acid-induced contraction
• OGR1 knockout in murine ASM inhibits acid-induced contraction
Mixed bag of effects (context-dependent)- What to do?

- Control pHo or pHi
- Block, activate, or bias GPCR signaling:
  a. Downstream signaling- usual suspect pathway inhibitors, COX inhibitors, antagonists of induced GPCR ligands, tyrphostins.
  b. At the receptor level for proton-sensing GPCRs; this quite hard given no ligands!
We have characterized a class of OGR1 ligands (allosteric modulators actually) and data to date that suggest we can bias OGR1 signaling
A subclass of (can’t tell you which because confidential!) regulate OGR1-mediated Ca\textsuperscript{2+} mobilization.
Some ligands appear balanced (L), some biased (S)
S stimulates Gs/ PKA but not Gq/Ca2+
HA OGR1 HEK stimulations

50μM L and 22
Stimulated for 10 min in HBSS
3/26/13
Characterizing newly discovered small molecule OGR1 ligands and their capacity for biasing Gs vs Gq signaling (Gs bias means bronchodilation!)

a. Human ASM cells and tissue
b. Guinea pig model of airway regulation by acid
c. OGR1-/- mouse
Contributors

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