Lecture 4 - Biased agonism

Sudarshan Rajagopal
Departments of Medicine and Biochemistry
sudarshan.rajagopal@duke.edu
Smith and Rajagopal, *Journal of Biological Chemistry* 2016
Smith and Rajagopal, *Journal of Biological Chemistry* 2016
What Effects can a Biased Agonist have on Physiology?

D.G. Soergel et al. / PAIN 155 (2014) 1829–1835
What Effects can a Biased Agonist have on Physiology?

D.G. Soergel et al. / PAIN 155 (2014) 1829–1835
Pluridimensional efficacy and bias at GPCRs

Structure-based discovery of opioid analgesics with reduced side effects

Nature volume 537, pages 185–190 (2016)
Structure based ligand discovery for the μOR

Discovery of a novel \( G_{i/o} \)-biased \( \mu \)OR agonist

Structure-guided optimization towards a potent biased μOR agonist
PZM21 is an analgesic with reduced on-target liabilities

Structural Basis of GPCR Biased Agonism
Similarities in the active GPCR conformation when binding G protein and arrestin
DEER spectroscopy to probe the effects of biased ligands at the $\text{AT}_1\text{R}$
Data from different pairs for each ligand
Conformations associated with different ligands

A closed

B A_{ocl1}

C A_{ocl2}

D A_{open}

E A_{open}

F

No ligand
Inverse agonist
AngII
\(\beta\)-arrestin-biased
Gq-biased

\(\beta\)-arrestin
Gq protein

Ligand-stabilized states
Transducer coupling
Spatial bias
Different modes of GPCR intracellular signaling
When trafficking and signaling mix: How subcellular location shapes G protein-coupled receptor activation of heterotrimeric G proteins
(A) Active GPCR

G Protein binding site available

(B) GPCR-β-arrestin ‘core’ conformation

G Protein binding site sterically occupied

(C) GPCR-β-arrestin ‘tail’ conformation

G Protein binding site available
Signaling from microdomains

Cell
Volume 185, Issue 7, 31 March 2022, Pages 1130-1142.e11
Endosomal signaling by non-GPCRs
Review of cytokine receptor trafficking

Clathrin-mediated endocytosis (CME) vs. Clathrin-independent endocytosis (CIE)

Caveolins, RhoA, Endophilins vs. Flotillin, Cdc42, Arf6

Plasma membrane

Recycling endosome

Internalized vesicle

Early endosome

Lysosome, Late endosome, Multivesicular body (MVB)

Colored symbols:
- receptor
- ligand
- dynamin
- clathrin
- actin filaments
- lipid raft

Cytokine & Growth Factor Reviews 32 (2016) 63–73
Endosomal signaling
Intracrine signaling by VEGF
Endocytosis can promote or inhibit specific RTK signaling
GPCR Endosomal Signaling
Modified from Dean Staus
Cellular trafficking of βarr2-GFP with the β2AR, V2R, and β2AR-V2R and V2R-β2AR chimeras.

The American Society for Biochemistry and Molecular Biology, Inc.
Colocalization of βarr2-GFP with the internalized β2AR, V2R, and β2AR-V2R and V2R-β2AR chimeras.

- **β2AR**
  - Receptor
  - βarr2-GFP
  - Overlay

- **β2AR-V2R chimera**
  - Receptor
  - βarr2-GFP
  - Overlay

- **V2R**
  - Receptor
  - βarr2-GFP
  - Overlay

- **V2R-β2AR chimera**
  - Receptor
  - βarr2-GFP
  - Overlay


The American Society for Biochemistry and Molecular Biology, Inc.
Phosphorylation of the agonist-activated β2AR, V2R, and β2AR-V2R and V2R-β2AR chimeras.

β-Arrestin binding site is located in the ear domain of β2-adaptin.
Overexpression β2-adaptin C-terminal subdomain inhibits the agonist-mediated internalization of β2AR.
Internalized AT$_1$R colocalizes with β-arrestin and pERK

**a**  
<table>
<thead>
<tr>
<th></th>
<th>HA-AT1αR</th>
<th>GFP-βarrestin 2</th>
<th>Overlay</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ang II</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**b**  
<table>
<thead>
<tr>
<th></th>
<th>GFP-βarrestin 2</th>
<th>RFP-ERK2</th>
<th>Overlay</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ang II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ang II</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
β-arrestin “signalosomes”
Effects of siRNA-suppressed β-arrestin2 (βarr2) expression on the kinetic pattern of ERK1/2 activation following stimulation of the AT1A receptor.

Effects of silencing β-arrestin2 (βarr2) expression on the subcellular distribution of phospho-ERK1/2 following different periods of stimulation of the AT1A receptor.

Time-dependent subcellular distribution of phospho-ERK1/2 and β-arrestin2 (βarr2)-RFP after stimulation of the AT1A receptor.

The American Society for Biochemistry and Molecular Biology, Inc.
G protein endosomal signaling
Endosomal cAMP generation

**PTHrP1-36**

Does not internalize

**PTH1-34**

internalizes

Inhibiting endocytosis decreases cAMP
Nb80–GFP detects activated $\beta_2$-ARs in the plasma membrane and endosomes.
Nb80–GFP accumulates on β2-AR-containing endosomes after their formation.
Internalized $\beta_2$-ARs contribute to the acute cAMP response.
GPCR Megaplexes as a Source for Endosomal GPCR Signaling?

CryoEM structure of the Megaplex
Different spatial and temporal patterns
Gβγ: the forgotten signaling molecule

Masuho et al., 2021, Cell Systems 12, 1–14
Gβγ can signal at specific subcellular locations

Masuho et al., 2021, *Cell Systems* 12, 1–14
Receptor signaling from other locations

If different ligands promote signaling from different locations, we refer to this as “location bias”
mGlu5 expressed on the nuclear membrane

**A** Wild Type mGlu5

**B** Mutant F767S mGlu5

**C** Isolated Nuclei

**D** Isolated Nuclei

**E** T PM N

**F** T PM N

mGlu5, Na⁺ K⁺ ATPase, Lamin B₂
And couples to nuclear Gq
Quis (permeant) vs DHPG (impermeant) – transcriptional changes in neurons

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 8, pp. 5412–5425, February 17, 2012
Proposed model of cell surface and intracellular mGlu5 receptor activation by glutamate.

Yuh-Jiin I. Jong et al. Mol Pharmacol 2014;86:774-785
GPCRs in the Golgi
β1AR signaling from Golgi
Golgi pool is distinct from endosomal pool

doi: 10.1038/nchembio.2389
Activates Gs at the Golgi

doi: 10.1038/nchembio.2389
Rapamycin induced recruitment of Nb80 blocks Gs signaling

![Diagram of β₁AR signaling pathways](image)

**Graphs:**
- **PM-targeted:**
  - Blue bar: Dobutamine (10 μM)
  - Cyan bar: Dobutamine (10 μM) + rap
  - Red bar: Dobutamine (10 μM) + rap + solatol (100 μM)
  - Luminescence (% of max Fsk)

- **Golgi-targeted:**
  - Blue bar: Dobutamine (10 μM)
  - Cyan bar: Dobutamine (10 μM) + rap
  - Red bar: Dobutamine (10 μM) + rap + solatol (100 μM)
  - Luminescence (% of max Fsk)

**Doi:** 10.1038/nchembio.2389
OCT3 transporter needed for impermeant ligands to get to the Golgi
Different effects of membrane permeant and impermeant antagonists

doi: 10.1038/nchembio.2389
Location-encoded signaling
β2AR signaling from endosomes

(a) 1 μM Iso vs. 1 μM Iso + Dyngo

(b) 10 nM Iso vs. 10 nM Iso + Dyngo
Endosomal signaling regulates transcription
Optogenetic activation of cAMP

*PCK1 expression

cAMP signal

doi: 10.1038/nchembio.1665
Temporal Bias

Nature Communications volume 7, Article number: 10842 (2016)
Differentiating the effects of spatial and temporal bias at the PTH1R
Characterization of a Gs-biased PTH analog generated by amino acid isomerization
Location bias of PTH7d signaling

(A) cAMP (% of Fsk) over time with different ligands: LA-PTH, PTH7d, and PTHWT.

(B) cAMP (after ligand washout) with PTHWT, LA-PTH, and PTH7d, and with antagonist PTH7d + antagonist.

(C) PTHSEP (ΔF/F0, % of max) over time with different ligands: LA-PTH, PTHWT, and PTH7d.

(D) Nuclear cAMP (normalized) and Nuclear PKA activity (normalized) over time with LA-PTH and PTH7d.
Molecular changes induced by PTH7d
Differential pharmacological actions of PTH7d, PTHWT, and LA-PTH in mice
Proposed model for location bias in cAMP and PTHR pharmacology