A BRIEF HISTORY OF G-PROTEIN COUPLED RECEPTORS

Nobel Lecture
Stockholm University
December 8, 2012

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G-Protein Coupled Receptors (GPCRs) 
Seven Transmembrane Receptors

- ~ 200 functionally known receptors
- ~ 600 functionally unassigned receptors (orphan)
- Hundreds of sensory (taste and smell) and hormone receptors
- Account for about 60% of all prescription drugs
- Examples: $\alpha$ and $\beta$-Adrenergic Receptor Blockers and Agonists, Serotonin Receptor Blockers and Agonists, Histamine Receptor H1 and H2 Blockers, Opioid Receptor Blockers and Agonists
A Brief History of Receptors

1900 – 1910 Early Ideas

J.N. Langley (1852-1926)

a) studied the actions of adrenaline and antagonistic drug pairs
    (nicotine, curare) – skeletal muscle
    (pilocarpine, atropine) – submandibular gland

b) “receptive substance”

“So we may suppose that in all cells two constituents at least are to be distinguished, a chief substance, which is concerned with the chief function of the cell as contraction and secretion, and receptive substances which are acted upon by chemical bodies and in certain cases by nervous stimuli. The receptive substance affects or is capable of affecting the metabolism of the chief substance” (Journal of Physiology 33, 374-413, 1905)
Early Skepticism

H.H. Dale (1875-1968)

“It is a mere statement of fact to say that the action of adrenaline picks out certain such effector-cells and leaves others unaffected; it is a simple deduction that the affected cells have a special affinity of some kind for adrenaline; but I doubt whether the attribution to such cells of “adrenaline-receptors” does more than re-state this deduction in another form.” (Transactions of the Faraday Society 39, 319-322, 1943)
A Brief History of Receptors

Later Skepticism

1973  R. Ahlquist  "...This would be true if I were so presumptuous as to believe that \( \alpha \) and \( \beta \) receptors really did exist. There are those that think so and even propose to describe their intimate structure. To me they are an abstract concept conceived to explain observed responses of tissues produced by chemicals of various structure"

1970-Present

The Molecular Era

1970’s Radioligand Binding ➔ Receptor Regulation

➔ Theories of receptor action
guanine nucleotide effects,
high & low affinity states
➔ Receptor subtypes

Vol. 60, No. 2, 1974

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
STEREOSPECIFIC [3H](-)-ALPRENOLOL BINDING SITES, ß-ADRENERGIC
RECEPTORS AND ADENYLYL CYCLASE

Robert J. Lefkowitz, Chhabirani Mukherjee, Michael Coverstone and Marc G. Caron
Division of Cardiology, Department of Medicine and Department of Biochemistry
Duke University Medical Center, Durham, North Carolina 27710

Alpha-Adrenergic Receptor Identification by [3H]Dihydroergocryptine Binding

LEWIS T. WILLIAMS

21 MAY 1976

791

ROBERT J. LEFKOWITZ

SCIENCE, VOL. 192
Allosteric Regulation of Receptors by G Proteins

A Ternary Complex Model Explains the Agonist-specific Binding Properties of the Adenylate Cyclase-coupled β-Adrenergic Receptor*

Received for publication, November 14, 1979, and in revised form, March 18, 1980

Andre De Lean,†‡ Jeffrey M. Stadel, and Robert J. Lefkowitz¶
From the Howard Hughes Medical Institute Laboratory, Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710
Isolation of Adrenergic Receptors

BIOSPECIFIC AFFINITY CHROMATOGRAPHY SUPPORTS FOR PURIFICATION OF ADRENERGIC RECEPTORS

(-) ALPRENOLOL

A-55414

SKF-101253

RECEPTOR SPECIFICITY

β₁ & β₂ AR

α₁ AR

α₂ AR

ADRENERGIC RECEPTORS

β₂

HAMSTER LUNG

α₁

SMOOTH MUSCLE CELL

α₂

HUMAN PLATELET

94K →

67K →

45K →

30K →

20K →

94K →

67K →

45K →

30K →

20K →

[²⁵] PABC [²⁵] PROTEIN

[²⁵] APDO [²⁵] PROTEIN

[³H] POB [²⁵] PROTEIN

[²⁵] PROTEIN
Reconstitution of β-adrenergic receptors in lipid vesicles: Affinity chromatography-purified receptors confer catecholamine responsiveness on a heterologous adenylate cyclase system

(octyl glucoside/Sepharose-alprenolol)

Richard A. Cerione*, Berta Strulovic*, Jeffrey L. Benovic†, Catherine D. Strader*, Marc G. Caron*,†, and Robert J. Lefkowitz*†

Howard Hughes Medical Institute, Departments of *Medicine (Cardiology), †Biochemistry, and ‡Physiology, Duke University Medical Center, Durham, North Carolina 27710

Communicated by Henry A. Lardy, May 2, 1983

Communication

The Journal of Biological Chemistry
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Printed in U.S.A.

Reconstitution of a Hormone-sensitive Adenylate Cyclase System

THE PURV β-ADRENERGIC RECEPTOR AND GUANYLIC NUCLEOTIDE REGULATORY PROTEIN CONFER HORMONE RESPONSIVENESS ON THE RESOLVED CATALYTIC UNIT

(Received for publication, April 18, 1984)


Reconstitution Assay

Donor Cell + Xenopus Acceptor Cell

Plasma Membrane

+ Octyl Glucoside

+ Phospholipid Vesicles

Incubation

Detergent removal

Centrifugation

Add Xenopus Erythrocytes + Phospholipids

Polystyrene Glycol (35°C)

Dilute with buffer

Hybrid Cell

Prepare membranes
Cloning of the gene and cDNA for mammalian $\beta$-adrenergic receptor and homology with rhodopsin

Richard A. F. Dixon*, Brian K. Kobilka†,
David J. Strader‡, Jeffrey L. Benovic†,
Henrik G. Dohlman†, Thomas Frielle‡,
Mark A. Bolanowski†, Carl D. Bennett§, Elaine Rands*,
Ronald E. Diehl*, Richard A. Mumford‡, Eve E. Slater‡,
Irving S. Sigal*, Marc G. Caron†, Robert J. Lefkowitz†
& Catherine D. Strader‡

Departments of *Virus and Cell Biology Research and §Medicinal Chemistry, Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania 19486, USA
† Howard Hughes Medical Institute, Department of Medicine, Biochemistry and Physiology, Duke University Medical Center, Durham, North Carolina 27710, USA
‡ Department of Biochemistry and Molecular Biology, Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey 07065, USA
TIPS Reviews

β-Adrenergic receptors and rhodopsin: shedding new light on an old subject

Robert J. Lefkowitz, Jeffrey L. Benovic, Brian Kobilka and Marc G. Caron

Biochemistry

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Volume 26, Number 10
May 19, 1987

Perspectives in Biochemistry

A Family of Receptors Coupled to Guanine Nucleotide Regulatory Proteins

Henrik G. Dohlman, Marc G. Caron, and Robert J. Lefkowitz*

Howard Hughes Medical Institute, Departments of Medicine, Physiology, and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

Received January 14, 1987; Revised Manuscript Received February 26, 1987
Regions of the Receptor Involved in Ligand & G Protein Binding

Site-directed Mutagenesis of the Cytoplasmic Domains of the Human \( \beta_2 \)-Adrenergic Receptor

LOCALIZATION OF REGIONS INVOLVED IN G PROTEIN-RECEPTOR COUPLING*

(Received for publication, April 20, 1988)

Brian F. O’Dowd‡§, Mark Hnatowich‡§¶, John W. Regan‡§, W. Mark Leader‡, Marc G. Caron‡¶, and Robert J. Lefkowitz‡§

From the Departments of ‡Medicine, §Biochemistry, and ¶Cell Biology, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina 27710

Research Articles

Chimeric \( \alpha_2-,\beta_2 \)-Adrenergic Receptors: Delineation of Domains Involved in Effector Coupling and Ligand Binding Specificity

Brian K. Kobilka, Tong Sun Kobilka, Kiefer Daniel, John W. Regan, Marc G. Caron, Robert J. Lefkowitz
Chimeric Receptors

Wild Type Receptors

\[ \alpha_2 \text{AR} \]

\[ \beta_2 \text{AR} \]

Effector System:

\[ \text{Gi} \rightarrow \downarrow \text{AC} \]

\[ \text{Gs} \rightarrow \uparrow \text{AC} \]

Antagonists:

Yohimbine, Cyanopindolol, Alprenolol

Agonists:

PAC, EPI, ISO, ISO, EPI, PAC

Human \( \beta_2 \)-Adrenergic Receptor

Extracellular

Intracellular
Constitutively Active Mutant Receptors

DISEASES CAUSED BY MUTATIONS OF HEPTAHELICAL RECEPTORS

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Disease</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodopsin</td>
<td>Cong. night blindness</td>
<td>Aut. dom.</td>
</tr>
<tr>
<td>LH</td>
<td>Familial male precocious puberty</td>
<td>Aut. dom.</td>
</tr>
<tr>
<td>TSH</td>
<td>Sporadic hyperfunctional thyroid nodules</td>
<td>Somatic</td>
</tr>
<tr>
<td>TSH</td>
<td>Familial nonautoimmune hyperthyroidism</td>
<td>Aut. dom.</td>
</tr>
<tr>
<td>CaR</td>
<td>Familial hypoparathyroidism</td>
<td>Aut. dom.</td>
</tr>
<tr>
<td>PTH/PTHrP</td>
<td>Jansen metaphyseal chondrodysplasia</td>
<td>Aut. dom.</td>
</tr>
<tr>
<td>FSH</td>
<td>Gonadotropin-independent spermatog.</td>
<td>Aut. dom.</td>
</tr>
</tbody>
</table>
Universal Mechanism of Receptor Regulation: Desensitization

\[
\beta_2 \text{ Adrenergic Receptor}
\]

- **Graph:** The graph shows the concentration of cAMP over time in response to the activation of the \( \beta_2 \) adrenergic receptor. The graph indicates a rapid increase in cAMP levels followed by a decrease, demonstrating desensitization.

- **Axes:**
  - **Y-axis:** cAMP (A.U.)
  - **X-axis:** Time (Seconds)

- **Data Points:**
  - The graph includes data points labeled as 1 µM Iso, indicating the addition of isoproterenol (Iso) at time 0, which triggers the release of cAMP.

- **Legend:**
  - 100 nM AngII
  - Angiotensin 1A Receptor

This graph illustrates the desensitization process in the context of receptor regulation, highlighting the dynamic changes in cAMP levels over time.
Communication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Printed in U.S.A.

Catecholamine-induced Desensitization of Turkey Erythrocyte Adenylate Cyclase

STRUCTURAL ALTERATIONS IN THE β-ADRENERGIC RECEPTOR REVEALED BY PHOTOAFFINITY LABELING*

(Received for publication, February 22, 1982)

Jeffrey M. Stadel, Ponnal Nambi, Thomas N. Lavin, Sarah L. Heald, Marc G. Caron, and Robert J. Lefkowitz

From the Howard Hughes Medical Institute, Departments of Medicine (Cardiology) and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

Fig. 3. Effect of propranolol on agonist promoted-altered mobility of β-adrenergic receptor polypeptides.

Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 3173–3177, June 1983
Biochemistry

Catecholamine-induced desensitization of turkey erythrocyte adenylate cyclase is associated with phosphorylation of the β-adrenergic receptor

(protein kinase/refractoriness/β-adrenodinosine 3',5'-cyclic monophosphate/photoaffinity labeling)

Jeffrey M. Stadel*, Ponnal Nambi, Robert G. L. Shorr*, Diane F. Sawyer, Marc G. Caron, and Robert J. Lefkowitz†
β-Adrenergic receptor kinase: Identification of a novel protein kinase that phosphorylates the agonist-occupied form of the receptor

(S49 lymphoma cells/kin- mutant/purification/desensitization/adenylate cyclase)

JEFFREY L. BENOVIC*, RUTH H. STRASER*, MARC G. CARON*, and ROBERT J. LEFKOWITZ*

Howard Hughes Medical Institute, Departments of *Medicine, *Biochemistry, and +Physiology, Duke University Medical Center, Durham, NC 27710

β-Adrenergic Receptor Kinase: Primary Structure Delineates a Multigene Family

JEFFREY L. BENOVIC,* ANTONIO DEBLASI,† W. CARL STONE, MARC G. CARON, ROBERT J. LEFKOWITZ

(1 OCTOBER 1989)

The receptor kinase family: Primary structure of rhodopsin kinase reveals similarities to the β-adrenergic receptor kinase

(guanine nucleotide-binding protein-coupled receptors/desensitization/serine/threonine protein kinase/polymerase chain reaction)

WULFING LORENZ*, JAMES INGLESE††, KRZYSZTOF PALCZEWISKI††, JAMES J. ONORATO‡‡, MARC G. CARON*†, and ROBERT J. LEFKOWITZ*†

*Howard Hughes Medical Institute, Departments of *Medicine, ††Cell Biology, ‡‡Biochemistry, Duke University Medical Center, Box 3821, Durham, NC 27710; and ‡‡R. S. Dow Neurological Sciences Institute of Good Samaritan Hospital and Medical Center, Portland, OR 97209

Contributed by Robert J. Lefkowitz, July 1, 1991

G Protein-Coupled Receptor Kinases

ROBERT J. LEFKOWITZ

Departments of Medicine and Biochemistry
and the Howard Hughes Medical Institute
Duke University Medical Center
Durham, North Carolina 27710
The G Protein-Coupled Receptor Kinases (GRKs)

Serine/Threonine Kinases

3 classes:

GRK1 (Rhodopsin Kinase)
GRK7

GRK2 (bARK1)
GRK3 (bARK2)

GRK4
GRK5
GRK6

Something is Missing: **Discovery of β-arrestins**

- Purified βARK (GRK2) loses ability to desensitize isolated β2-AR (Benovic et al ‘85,’86)

- Abundant retinal protein, “48 K protein” or “S Antigen” works with rhodopsin kinase to deactivate rhodopsin renamed arrestin (Kuhn, et al ’87)

- “48 K protein” at high concentrations restores ability of βARK to desensitize β2-AR – (Benovic et al ’87)

*Proc. Natl. Acad. Sci. USA*
Vol. 84, pp. 8879–8882, December 1987

Biochemistry

Functional desensitization of the isolated β-adrenergic receptor by the β-adrenergic receptor kinase: Potential role of an analog of the retinal protein arrestin (48-kDa protein)

J. L. Benovic*, H. Kühn†, I. Weyand†, J. Codina†, M. G. Caron*, and R. J. Lefkowitz*
Discovery of β-arrestins

- S antigen (48 kDa protein) cloned - (Shinohara et al '87)

Primary and secondary structure of bovine retinal S antigen (48-kDa protein)

T. Shinohara*, B. Dietzschold†, C. M. Craft*, G. Wistow+, J. J. Early†, L. A. Donoso‡, J. Horwitz‡, and R. Tao†

- β-arrestin1 cloned – (Lohse et al ’90)

- β-arrestin2 cloned – (Attramadal et al ’92)
### The Arrestins

<table>
<thead>
<tr>
<th>Arrestin 1</th>
<th>AKA</th>
<th>Distribution</th>
<th>7MSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Visual Arrestin)</td>
<td>Retinal rods</td>
<td>Rhodopsin</td>
<td></td>
</tr>
<tr>
<td>β-Arrestin 1</td>
<td>(Arrestin 2)</td>
<td>Ubiquitous</td>
<td>Most</td>
</tr>
<tr>
<td>β-Arrestin 2</td>
<td>(Arrestin 3)</td>
<td>Ubiquitous</td>
<td>Most</td>
</tr>
<tr>
<td>X Arrestin</td>
<td>(Arrestin 4)</td>
<td>Retinal cones</td>
<td>Opsins</td>
</tr>
</tbody>
</table>

Two Paradigms: Activation & Desensitization

- **Activation**: 
  - Agonist binds to the receptor, activating Gαs. 
  - Gαs stimulates AC, leading to increased cAMP. 
  - cAMP activates PKA, which can lead to cell response. 

- **Desensitization**: 
  - Agonist binds to the receptor, activating GRK2. 
  - GRK2 phosphorylates the receptor, leading to β-arrestin recruitment. 
  - β-arrestin internalizes the receptor, preventing further signaling. 
  - Desensitization indicates a decrease in cell response.
New Signaling Paradigm

- Agonist binding to G protein-coupled receptors (GPCRs)
- Activation of G proteins $G_\alpha$, $G_\beta\gamma$
- Second messenger production:
  - $cAMP$
  - $DAG$
  - $IP_3$
- MAP kinases (Src, Akt, Others)
- Cell survival / anti-apoptosis
- Cardiac contractility
- Chemotaxis
- Dopaminergic behaviors
- Desensitization pathway
  - $\beta$-arrestin
  - GRK
- Cell response: various biological outcomes

---

- $\alpha$-adrenergic receptors
- $\beta$-adrenergic receptors
- $\gamma$-adrenergic receptors
- Chemokine receptors
- Dopamine receptors
- G protein subunits
- MAP kinases
- $cAMP$
- $DAG$
- $IP_3$
- $\beta$-arrestin
- GRK
A “Biased Agonist” is a ligand which stabilizes a particular active conformation of a receptor thus stimulating some responses but not others. Seven transmembrane receptor ligands, for example, can be biased toward a particular G protein or β-arrestin. Mutated receptors can also be biased.

\[
\begin{align*}
A + R & \leftrightarrow AR^* \rightarrow \text{All Signaling} \\
A_1 \text{ (biased agonist 1)} + R & \leftrightarrow AR_1^* \text{ (G protein)} \\
A_2 \text{ (biased agonist 2)} + R & \leftrightarrow AR_2^* \text{ (β-arrestin)}
\end{align*}
\]
A Selective $\beta$-arrestin biased ligand at the AT$_{1A}$R

Full agonist (AngII)

$\beta_{ar}r$ EC$_{50} = 9.7$ nM

- G-protein Signal (IP1)
- B-arrestin Recruitment (PathHunter)
Quantitative, Global Phosphorylation Analysis of β-arrestin mediated Signaling

MAPK signaling

- ARAF
- MEKK1
- NIK
- ASK
- TAK
- MAP3K2
- MEK1
- PKD1
- MEK4/7
- TAB2
- PKN2
- ERK1/2
- JNK1
- p38
- STAT1
- IkBα
- c-Jun

AT1aR

- β-arrestin

Phosphorylation Regulation

- Interaction with β-arrestin

- Both

- Interactome

- phosphoproteome
A “biased ligand” at the AT$_{1A}$R signals only through β-arrestin

Violin & Lefkowitz, TiPS 2007
## Ligands which are biased toward either β-arrestin or G-Protein Signaling have Potential Therapeutic Benefit

<table>
<thead>
<tr>
<th>7TMR</th>
<th>Example</th>
<th>Direction of Bias</th>
<th>Advantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opioid Receptor</td>
<td>TRY420027</td>
<td>β-arrestin</td>
<td>Reduced side effects such as constipation, respiratory depression, lowered blood pressure, decreased tolerance, increased cardiac performance, antiapoptotic</td>
</tr>
<tr>
<td>G-Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Mu-opioid receptor desensitization by beta-arrestin-2 determines morphine tolerance but not dependence


### Selectively engaging β-arrestin at the AT1R reduces blood pressure and increases cardiac performance


### β-arrestin1 mediates nicotinic acid induced flushing, but not its antilipolytic effect


### Morphine side effects in beta-arrestin 2 knockout mice

Raehal KM, Walker JK, Bohn LM.

J Pharmacol Exp Ther. 2005 Sep;314(3):1195-201.

Ahn S, Kim J, Hara MR, Ren XR, Lefkowitz RJ.
