"Role for \( \beta_2 \)AR biased signaling to address the \( \beta \)-agonist controversy in asthma"

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Support provided by NHLBI & NIAID:
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New perspectives on the β-agonist controversy in asthma

Outline

- Asthma: signs and symptoms
- Beta-2-adrenergic receptors (β2AR) and β-agonists
- The β-agonist controversy
- Results from animal models
- Dual signalling pathways of β2AR
- New therapeutic opportunities
Dual β₂AR Signaling Pathways

β-agonist → β₂AR → β-arrestin

Gs → AC → cAMP → reduced Ca⁺⁺ → Reduce ASM contraction

MAPK → Promote inflammation & AHR

Dickey, Walker, Hanania, Bond. COPhar 10(3): 254-9, 2010
Asthma Review
Asthma is a chronic inflammatory disorder

- Influx of inflammatory cells (Th2 & Eos)
- Increased mucin production and secretion
- Airway remodeling
- Airway hyperresponsiveness (twitchy)
- Reversible airway obstruction
- Cough, wheeze, shortness of breath
FEV1 and Methacholine PC$_{20}$

FEV1: forced expired volume in 1 second

PC$_{20}$ methacholine:
- Airway hyperresponsiveness indicator
- Baseline FEV1 is measured
- Inhale aerosolized escalating doses of MCh, measure FEV1
- MCh dose at which FEV1 has declined 20% from baseline
- The more hyperresponsive a patient is, the lower the PC$_{20}$
Murine Asthma Model – Methods & Measurements

OVA = ovalbumin
Airway hyperresponsiveness predicts asthma severity and future risk in humans.

American Thoracic Society Documents

Standardizing Endpoints for Clinical Asthma Trials and Clinical Practice

Assessment of murine lung mechanics outcome measures: alignment with those made in asthmatics

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The Controversy Surrounding β_{2}AR agonist use in Asthma

Review β_{2}AR
β₂-adrenergic receptor (β₂AR)

• ~ 200 functionally known receptors
• ~ 600 functionally unassigned receptors (orphan)
• Hundreds of sensory (taste and smell) and hormone receptors
• Targets for ~ 40% of all prescription drugs
### The Arrestin Family

<table>
<thead>
<tr>
<th>Name</th>
<th>Length</th>
<th>% Identity</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-arrestin 1</td>
<td>418 AA</td>
<td>100</td>
<td>Ubiquitous</td>
</tr>
<tr>
<td>β-arrestin 2</td>
<td>420 AA</td>
<td>95</td>
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</tr>
<tr>
<td>Arrestin</td>
<td>404 AA</td>
<td>66</td>
<td>Retina</td>
</tr>
</tbody>
</table>

βarrestin proteins
β₂-adrenergic receptor (β₂AR)
Classic Paradigm of 7TMR Signaling

Shenoy & Lefkowiz, Science, 2005
New Paradigm of β-Arrestin function: β-Arrestin Acts as a Signaling Molecule

Shenoy & Lefkowiz, Science, 2005
7TMR Signal Transduction

**NEW PARADIGM**

- Agonist
- 7TMR
- Gα
- Desensitization
- MAPKs
- Tyrosine Kinases
- AKT
- PI3 Kinases
- NFκB pathway
- cAMP
- DAG
- IP3
- 2nd messengers
- Cell Response

**NEWER PARADIGM**

- Agonist
- 7TMR
- Gα
- Desensitization
- MAPKs
- Tyrosine Kinases
- AKT
- PI3 Kinases
- NFκB pathway
- cAMP
- DAG
- IP3
- 2nd messengers
- Cell Response

**β-arrestin Signaling**

- Cell survival
- Anti-apoptosis
- Others
- Cardiac function
- Chemotaxis
- Dopaminergic behaviors
- Cancer

Shenoy & Lefkowiz, Science, 2005
β2AR-mediated bronchodilation

Figure credit to themedicalbiochemistrypage.org
The Controversy Surrounding $\beta_2$AR use in Asthma
β-agonist treatment in asthma:
SABAs and LABAs

**Short Acting Beta Agonists (SABAs)**
- used for immediate relief of bronchospasm
- Rapid onset of action, but short duration
- albuterol – ie., ventolin, bricanyl

**Long Acting Beta Agonists (LABAs)**
- ~12 h effect
- Inhaled twice daily (in combination with ICS)
- Bronchodilation
- BPE – reduces bronchoconstriction
- Salmeterol (Advair) & Formoterol (Symbicort)
Deleterious Effects of Chronic Inhaled $\beta_2$-agonist use in Asthmatics (Short-acting)

Epidemic of asthma deaths followed introduction of:
- high-dose isoproterenol in the UK, 1960s
- fenoterol in New Zealand, late 1970s

Regular use of short-acting inhaled $\beta_2$-agonists:
- increases airway responsiveness to allergen
- increases asthma morbidity
- reduces effectiveness of “rescue” $\beta_2$-agonist

Short-acting $\beta_2$-agonist for RESCUE ONLY
Controversy surrounding LABA use

- concerns about mortality risk
  - Salmeterol Nationwide Surveillance Study (1993)
  - Salmeterol Multicenter Asthma Research Trial (2003)

- functional \( \beta_2 \)AR tachyphylaxis (Newnham et al., 1994; 1995; Grove and Lipworth, 1995),

- deterioration of asthma control (Sears, 2002; Salpeter et al., 2006)

- death (Stolley and Schinnar, 1978; Spitzer et al., 1992; Pearce et al., 1995).

- no adverse events of chronic beta-agonism (Drazen et al., 1996; Dennis et al., 2000; Bateman et al., 2008)
Controversy Surrounding LABA use in Asthma

- Results of FDA randomized, double-blind, controlled clinical trials - LABA + ICS avoids adverse asthma events

Stempel et al., 2016 NEJM vol 374:pg 1822
Stempel et al., 2016 NEJM vol 375:pg 840

The four studies supported the decision to remove the Boxed WARNING … retain a Warning and Precaution related to the increased risk of asthma-related death when LABAs are used without an ICS to treat asthma.
Beta-blocker goes on trial as asthma therapy

Alison Abbott

A drug for lowering high blood pressure is to be tested as a treatment for asthma despite warnings on its packaging that it should not be prescribed to asthmatics.

The US Food and Drug Administration approved the trial on nadolol, one of a group of drugs called beta-blockers, because some animal tests have suggested it could give long-term relief from the symptoms of asthma.

The approval for the clinical trial is seen by many in the field as a vindication for pharmacologist Richard Bond of the University of Houston in Texas. Bond has been pushing for years for beta-blockers to be used to treat asthma, despite claims by colleagues that the idea is counterintuitive or even dangerous.

“Every medical student learns never to prescribe a beta-blocker to an asthmatic, because it would have a potentially fatal effect,” says Clive Page, a pharmacologist and asthma expert at King’s College London.

To say a drug may not kill you if you take enough of a second drug is hardly a ringing endorsement for the first drug.”

- Richard Bond
Dual $\beta_2$AR Signaling Pathways

- **$\beta$-agonist**
- **$\beta$-arrestin**
- **Gs**
- **AC**
- **MAPK**
- **cAMP**
- **Promote inflammation & AHR**
- **Reduced Ca**
- **Reduce ASM contraction**

Dickey, Walker, Hanania, Bond. COPhar 10(3): 254-9, 2010
Does $\beta_2$AR Activation Drive the Asthma Phenotype?
Does $\beta_2$AR Activation Drive the Asthma Phenotype?

1. Genetic deletion of $\beta_2$AR: effect on asthma phenotype

$\beta_2$AR-KO mice
β2-AR gene disruption reduces airway inflammation

Nguyen L P et al. PNAS 2009;106:2435-2440
β2-AR gene disruption reduces mucin content in the airway epithelium.

Nguyen L P et al. PNAS 2009;106:2435-2440
β2-AR gene disruption reduces airway hyperresponsiveness

Nguyen L P et al. PNAS 2009;106:2435-2440
Does $\beta_2$AR Activation Drive the Asthma Phenotype?

Summary of murine data

1. Mice that lack $\beta_2$AR do not develop the asthma phenotype
Does $\beta_2$AR Activation Drive the Asthma Phenotype?

2. Genetic epinephrine deletion: effect on asthma phenotype

PNMT-KO mice
Loss of endogenous epinephrine prevents airway inflammation

Loss of endogenous epinephrine prevents elevated airway mucin volume

Loss of endogenous epinephrine prevents airway hyperresponsiveness

Does $\beta_2$AR Activation Drive the Asthma Phenotype?

Summary of murine data

1. Mice that lack $\beta_2$AR do not develop the asthma phenotype

2. Mice that lack epinephrine do not develop the asthma phenotype
Does $\beta_2$AR Activation Drive the Asthma Phenotype?

3. Exogenous Administration of $\beta_2$AR-agonist: effect on asthma phenotype

Chronic Salbutamol Treatment
Chronic administration of albuterol: effect on asthma phenotype

Chronic OVA +/- Albuterol Treatment

Ova Aerosol Inhalation

+ Oropharyngeal Albuterol

Lavage and Histology – mucin and airway inflammation

Lin et al., BJP (2012):165 2365-77
Chronic albuterol treatment promotes airway inflammation and mucin production

Lin et al., BJP (2012):165 2365-77
Chronic albuterol treatment promotes airway hyperresponsiveness

Lin et al, BJP 2012, 165 2365-77
1. Mice that lack β2AR do not develop the asthma phenotype
2. Mice that lack epinephrine do not develop the asthma phenotype
3. Mice chronically treated with albuterol display a more severe asthma phenotype

Summary of murine data

Does β2AR Activation Drive the Asthma Phenotype? YES! (at least in mice)
β2AR Dual Signaling Pathways
Dual β2AR Signaling Paradigm

β-agonist

Epinephrine-KO

β2AR-KO

βarr2-KO

β-arrestin

Gs

AC

MAPK

cAMP

reduced Ca++

Pro-inflammatory
Pro-asthmatic

Relief from Bronchospasm

Does β-arrestin-dependent signaling drive the asthma phenotype?
β-Arrestin-2 regulates the development of allergic asthma

Julia K.L. Walker,¹ Alan M. Fong,¹ Barbara L. Lawson,¹ Jordan D. Savov,¹ Dhavalkumar D. Patel,¹ David A. Schwartz,¹ and Robert J. Lefkowitz¹,²

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²Howard Hughes Medical Institute. Duke University Medical Center, Durham, North Carolina, USA


Wild-type

β-arrestin-2-KO

Walker *et al.*, *JCI*, 2003
Lavage total cells, lymphocytes, eosinophils and \( T_H^2 \)-cytokines were significantly elevated in OVA-treated WT mice but not \( \beta \)-arrestin-2-KO mice.

Walker et al., JCI, 2003
Is βarr2 required for Perpetuation of the Asthma Phenotype?

“Asthma Resolution Study”
Asthma Resolution Study

Chen et al., AJRCMB 2015
Asthma Resolution Study

Results for whole lung are shown here.

TMX treatment significantly reduces βarr2 expression.

Chen et al., AJRCMB 2015
Asthma Resolution Study

OVA-induced airway responsiveness to MCh requires βarr2 expression

Chen et al., AJRCMB 2015
βarr2 expression plays a role in OVA-induced lung inflammation.

Chen et al., AJRCMB 2015
βarr2 expression may/may not play a role in OVA-induced airway inflammation and mucin production.

Chen et al., AJRCMB 2015
Does $\beta$arr-dependent signaling drive the Asthma Phenotype?

Summary of murine data

1. Mice that lack $\beta$arr2 do not develop the asthma phenotype despite being sensitized to OVA

2. Mice that have an established asthma phenotype lose AHR when $\beta$arr2 expression is significantly reduced

$\beta$arr2 mediates both the development and the perpetuation of the asthma phenotype*
Does the β2AR-mediated β-arrestin-dependent signaling pathway drive the asthma phenotype?
Does the β2AR-mediated βarr2 dependent signaling pathway drive the asthma phenotype?

**What Cell Types?**

- Airway Epithelial Cells?
- Airway Smooth Muscle Cells?
- TH2 cells?
Does the $\beta_2$AR-mediated $\beta$arr2 dependent signaling pathway drive the asthma phenotype?

What Cell Types?

Airway Epithelial Cells?

Airway Smooth Muscle Cells?

TH2 cells?
What Cell Types?
Airway Epithelial Cells?

Epinephrine Activation of the $\beta_2$-adrenoceptor is Required for IL-13-Induced Mucin Production in Human Bronchial Epithelial Cells.

Nour Al-Sawalha$^{1,3}$, Indira Pokkunuri$^1$, Ozozoma Omoluabi$^2$, Hosu Kim$^1$, Vaidehi J. Thanawala$^1$, Adrian Hernandez$^2$, , Richard A. Bond$^{1,2}$ and Brian J. Knoll$^{1,2}$,*

PLOS ONE | DOI:10.1371/journal.pone.0132559 July 10, 2015
Epinephrine-mediated activation of β2AR (and not β1AR) is required for IL-13 induced Mucin production in NHBE cells

Al-Sawahla et al PLOS ONE July 10, 2015
What Cell Types?
Airway Epithelial Cells?

β2AR activates ERK ½ signaling in NHBE. This activation is inhibited by FR180204

Al-Sawahla et al PLOS ONE July 10, 2015
Does the β2AR-mediated βarr2 dependent signaling pathway drive the asthma phenotype?

What Cell Types?
Airway Epithelial Cells?
Airway Smooth Muscle Cells?
TH2 cells?
βarr2 constrains β₂-AR signal transduction in Airway Smooth Muscle

βarrestin-2 constrains β2AR-mediated cAMP accumulation in human ASMCs

**Human Cell Culture Data**

- βarrestins are expressed in human trachealis.

- βarrestins functionally constrain Iso-induced cAMP accumulation in human ASM cells.

Deshpande et al., FASEB J, 2008.
β-arrestin-2 constrains β2AR-induced in vivo airway relaxation

- β-arrestin-2 functionally constrains β2AR-mediated, but not PGE2-mediated, ASM relaxation.

In vivo Data

Deshpande et al., FASEB J, 2008.
Biasing $\beta_2$AR Signaling Pathways

$\beta_2$AR-KO  $\rightarrow$  $\beta$-agonist  $\rightarrow$  Epinephrine-KO

$\beta$-agonist  $\rightarrow$  $\beta$arr2-KO  $\rightarrow$  $\beta$arrestin

$\beta$arr2-KO  $\rightarrow$  Gs  $\rightarrow$  AC  $\rightarrow$  cAMP  $\rightarrow$  reduced Ca$^{++}$

MAPK  $\rightarrow$  ???

Pro-inflammatory  Pro-asthmatic  Relief from Bronchospasm

What are biased ligands?
Receptor Theory for Dual $\beta_2$AR Signaling Pathways

Walker et al., British Journal of Pharmacology (2011) 163 18–28
Most (known) β2AR agonists are not biased – allosteric modulation?

Rajagopal, Ahn,…Lefkowitz, 2011, Mol Pharm, 80:367
Genetic Polymorphisms of $\beta_2$AR

ACUTE SABA - Arg/Arg $\uparrow$ bronchodilation
CHRONIC SABA – Arg/Arg deterioration symptoms

Ortega et al., 2015,8:9-22
Ortega…Bleecker 2007,27:665
Genetic Variants in β2AR

- Arg16 homozygotes (more common in Asians) showed increased bronchodilation to SABA (acutely), but loss of bronchodilatory effect when SABA administered chronically
- “… multiple clinical retrospective and prospective clinical trials were not able to identify significant differences in PEFR-responsiveness to LABA therapy between Gly16Arg genotypes.”
- 4th TM domain - (Thr^{164}Ile) reduced SABA and LABA affinity, decreased Gs signaling (more common in non-Hispanic whites)

Ortega 2015,8:9-22
Pharmacogenetic Genes for β2AR effect

Table 3 Pharmacogenetic candidate genes for β₂-adrenergic receptor response in asthma

<table>
<thead>
<tr>
<th>β₂-adrenergic receptor agonists drug classes</th>
<th>Gene</th>
<th>Associated loci</th>
<th>Study design</th>
<th>Response phenotype</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Short-acting beta agonists (albuterol)</td>
<td>CRHR2</td>
<td>rs7793837</td>
<td>Candidate gene study</td>
<td>Acute FEV₁ ; bronchodilation</td>
<td>58,64</td>
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<tr>
<td></td>
<td>ADCY9</td>
<td>rs2230739 (Ile²⁷²Met)</td>
<td>Candidate gene study</td>
<td>Acute FEV₁ ; bronchodilation</td>
<td>19,64</td>
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<td>ADRB2</td>
<td>rs1042713 (Gly¹⁶Arg)</td>
<td>Candidate gene study</td>
<td>Acute FEV₁ ; bronchodilation</td>
<td>48,85</td>
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<td>ARG1</td>
<td>rs2781659, rs2781667</td>
<td>Candidate gene study</td>
<td>Acute FEV₁ ; bronchodilation</td>
<td>9</td>
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<tr>
<td></td>
<td>ARG2</td>
<td>rs7140310, rs10483801</td>
<td>Candidate gene study</td>
<td>Acute FEV₁ ; bronchodilation</td>
<td>50</td>
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<td></td>
<td>NOS3</td>
<td>rs1799983 (Asp²⁹⁶Glu)</td>
<td>Candidate gene study</td>
<td>Acute FEV₁ ; bronchodilation</td>
<td>60–62,64</td>
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<td>THRβ</td>
<td>rs892940</td>
<td>Candidate gene study</td>
<td>Acute FEV₁ ; bronchodilation</td>
<td>61</td>
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<tr>
<td></td>
<td>SLC24A4</td>
<td>rs77441273 (Arg⁸⁸⁵Gln)</td>
<td>GWAS</td>
<td>Acute FEV₁ ; bronchodilation</td>
<td>63</td>
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<tr>
<td></td>
<td>SLC22A15</td>
<td>rs1281748, rs1281743</td>
<td>Admixture mapping</td>
<td>Acute FEV₁ ; bronchodilation</td>
<td>73</td>
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<tr>
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<td>SPATS2L</td>
<td>rs295137</td>
<td>Candidate gene study</td>
<td>Acute FEV₁ ; bronchodilation</td>
<td>59</td>
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<td>Long-acting beta agonists (salmeterol and formoterol)</td>
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<td>Candidate gene study</td>
<td>Long-term FEV₁ response</td>
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<td>ADRB2</td>
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<td>Candidate gene study</td>
<td>Long-term PEFR response</td>
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<td>No effect on PEFR response</td>
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<td>Candidate gene study</td>
<td>Genotype-stratified</td>
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<td>SLC24A4</td>
<td>rs77441273 (Arg⁸⁸⁵Gln)</td>
<td>GWAS</td>
<td>Preference for montelukast or LABA as add-on to ICS</td>
<td>59</td>
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<tr>
<td></td>
<td>SLC22A15</td>
<td>rs1281748, rs1281743</td>
<td>Admixture mapping</td>
<td>Preference for montelukast or LABA as add-on to ICS</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>SPATS2L</td>
<td>rs295137</td>
<td>Candidate gene study</td>
<td>Exacerbation requiring hospitalization</td>
<td>64</td>
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</table>

Notes: Biologic candidate genes are summarized by drug class, associated genetic loci (rs number and coding change, if relevant), study design, and response phenotype for which a pharmacogenetic association has been described. Reproduced from Ortega VE and Wechsler ME. *Curr Opin Allergy Clin Immunol*. 2013;13(4):399–409. Promotional and commercial use of the material in print, digital or mobile device format is prohibited without the permission from the publisher Lippincott Williams & Wilkins. Please contact journalpermissions@lww.com for further information.⁷⁰

Abbreviations: FEV₁, forced expiratory volume in 1 second; GWAS, genome-wide association studies; ICS, inhaled corticosteroids; LABA, long-acting beta agonist; PEFR, peak expiratory flow rate.
Genetic Polymorphisms of β2AR

Asthma pharmacogenetics and the development of genetic profiles for personalized medicine

Abstract: Human genetics research will be critical to the development of genetic profiles for personalized or precision medicine in asthma. Genetic profiles will consist of gene variants affecting therapeutic responsiveness, adverse responses, gene-gene interactions (LABA + ICS), drug metabolism, consider severity subgroups and appropriateness of response.
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