Regulation of the Actin Cytoskeleton in Human Airway Smooth Muscle Tone

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What is Asthma?

• Chronic lung disease that narrows airways
• Recurring wheezing, coughing, chest tightness and shortness of breath
Asthma’s Significance

- 25 million diagnosed and increasing
- Over 10 million outpatient visits and 40,000 hospitalizations
- 3,400 deaths each year
- Most common chronic illness among children

$44 Billion Dollar Problem


Current Forms of Treatment

• Long-term control: Inhaled Corticosteroids and Long-Acting $\beta$-Agonists
  – Safe for long term use when combined
  – Recent concerns with LABA
  – Fluticasone (Flonase), Mometasone (Nasonex)

• Quick relief for attacks: Short-Acting $\beta$-agonists
  – Cannot be used regularly
  – Albuterol (ProAir), Levalbuterol (Xopenex)

NIH: National Heart, Lung, and Blood Institute
β-agonists

- G-protein Coupled Receptor

Smooth Muscle Tone Regulation: $\beta_2$-adrenergic receptor and m3 muscarinic acetylcholine receptor

Problems with Long-Term Use

Desensitization
1. Phosphorylation of the receptor
2. Internalization of cell-surface receptors
3. Downregulation of production of new receptors

Problems with Long-Term Use

- Polymorphisms of the human $\beta_2$-adrenoreceptor
- Codon 16: 16% Homozygous Arg-Arg, 37% heterzygous Arg-Gly, 47% Homozygous Gly-Gly

Side Effects

• β-Agonist non-tissue specific signaling: increased heart rate, increased blood sugar, and hypokalemia that may invoke cardiac arrhythmias

• Inhaled corticosteroids reduce adrenocortical activity, increase the risk of cataracts, and do not work effectively in smokers
Smooth Muscle Contraction

- Cross-Bridge Cycle model well-established

- New evidence points to requirement of actin polymerization

New Therapeutics

• Target actin polymerization in airway smooth muscle

• Regulator of actin dynamics: HSP20
  – Downstream target of β-agonists
  – Phosphorylation at Serine 16 induces muscle relaxation
  – No effect on myosin light chain phosphorylation or intracellular Ca$^{2+}$
Alternative Molecular Targets: β-agonist Pathway

- Decreased intracellular Ca$^{2+}$
- Myosin Contraction
- Phosphorylation of Hsp20

Asthma Pathogenesis and ASM Relaxation

\[ \beta_2 - \text{Agonists} \]

\[ \beta_2-\text{AR} \]

Gs

AC

cAMP

PKA

P-HSP20

Peptide Mimetics

HSP20

P-HSP20

Actin depolymerization

ASM Relaxation

Chronic use Of $\beta_2$ – Agonists

PKA, $\beta$ARK phosphorylation

Inflammation

Genetic polymorphism

Desensitization

P-HSP20

Komalavilas 2012
Phospho-HSP20 Peptide

- YARAAARQARAWLRRApSAPLPGLK
- 13 amino acid sequence surrounding phosphorylated Serine 16 (red)
- Protein transduction domain from HIV TAT protein (blue)
- Caveolae-dependent internalization

Goal of the Study

• Determine the effects of P20 peptide on ASM relaxation and actin polymerization
• Determine the effects of P20 peptide on actin fiber disruption in human airway smooth muscle cells
Drugs

• Carbachol-Cholinergic agonist that binds to the m3 muscarinic acetylcholine receptor
• Isoproterenol-analog of epinephrine that acts as a β-agonist at the β₂-adrenergic receptor
• P20 peptide-phosphomimetic peptide
Figure 1: P20 peptide disrupts the formation of stress fibers by the contractile agonist carbachol
Muscle Bath

• Measures ring tension
Figure 2: P20 peptide inhibits contraction in pig ASM
Figure 3: ISO and P20 peptide decrease the F-actin pool in pig ASM stimulated with CCH
Figure 4: P20 peptide decreases migration in HASMCs
Figure 5: P20 peptide does not have negative effects on HASMC proliferation
Conclusions

• P20 peptide *inhibits* the formation of stress fibers in HASMCs
  – Previous studies found a loss of actin in other cell types

• P20 peptide can inhibit contraction of stimulated pig ASM
  – Previous studies found P20 peptide induces relaxation

• Together, suggests that actin is depolymerized prior to and during stimulation and prevents contraction
Conclusions

- P20 peptide dramatically increases the pool of G-actin, although only one trial
- Actin depolymerization by P20 peptide is sufficient to inhibit migration
- P20 peptide is not cytotoxic and will not promote hyperplasia of the airway according to cell studies
Further Directions

• More actin assay trials
  – Human lungs
• Dose response in cell experiments
• Animal models of asthma
Why is this important?

- Medicine is moving to become more personalized
- Genomic Therapeutics: SNPs such as codon 16
- Targeting the molecular basis of smooth muscle contraction
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