Mechanical-Energetic Coupling in Airway Smooth Muscle

Gary C. Sieck, Ph.D.
Department of Physiology & Biomedical Engineering
Mayo Clinic College of Medicine

Evolution of Academic Medical Centers

Symmorphosis Hypothesis - In evolution, no structure is formed or maintained unless it satisfies functional demands (Weibel & Taylor, 1981). Biological systems adhere to an ‘economy of design’ that can be applied to other complex systems.

- For academic medical centers, the capacities for basic science discovery, translational research and clinical practice should be matched.
Bedside to Bench Back to Bedside Approach

Identify clinical problems

Conduct targeted research

Apply research to clinical problems

Asthma affects 17 million people in the USA (~8% of the population). Characterized by airway smooth muscle hyperresponsiveness and remodeling - triggered by inflammation and mediated by inflammatory cytokines.

Asthma: Airways Hyperresponsiveness and Remodeling

Identify clinical problems

Conduct targeted research

Apply research to clinical problems

Dynamic Properties of the Airway

Airway smooth muscle contractile proteins are highly adaptive to intrinsic and extrinsic stresses and are embedded within a malleable cytoskeletal structure that is tethered to the extracellular matrix. The endoplasmic (sarcoplasmic) reticulum (ER/SR) and mitochondria are also highly dynamic motile structures within the cell.

Dynamic Properties of the Airway

Excitation-Contraction Coupling in Airway Smooth Muscle

Elevated $[Ca^{2+}]_i$

Calmodulin (CaM)

$Ca^{2+}$ -CaM

MLC Kinase

Cross-Bridge Cycling

Cross-Bridge Recruitment

$MLC_{ph}$ Phosphorylation

$MLC_{ph}$ Dephosphorylation / Relaxation

ATP

$Ca^{2+}$ -CaM

MLC Kinase

Cross-Bridge Cycling

Cross-Bridge Recruitment

$MLC_{ph}$ Phosphorylation

$MLC_{ph}$ Dephosphorylation / Relaxation

ATP
Intracellular Ca\textsuperscript{2+} Regulation in Airway Smooth Muscle

Spontaneous Ca\textsuperscript{2+} Transients (Sparks) in Airway Smooth Muscle

Excitation-Energy Coupling in Airway Smooth Muscle

Inflammatory Cytokines Enhance Intracellular Ca\textsuperscript{2+} Response in Airway Smooth Muscle
Ca\textsuperscript{2+} Sparks Reflect SR Ca\textsuperscript{2+} Release via RyR Channels in Airway Smooth Muscle Cells

- Ca\textsuperscript{2+} sparks are unaffected by altered Ca\textsuperscript{2+} influx.
- Incidence of Ca\textsuperscript{2+} sparks increases with an increase in the open probability of RyR channels.

Ca\textsuperscript{2+} Sparks Fuse into Larger Ca\textsuperscript{2+} Transients in Airway Smooth Muscle Cells

- Anything that increases open probability of RyR channels (e.g., cADPR) increases the incidence of larger Ca\textsuperscript{2+} transients.

ACh-Induces Propagating [Ca\textsuperscript{2+}] Oscillations in Airway Smooth Muscle Cells

- Larger Ca\textsuperscript{2+} transients propagate through the cell and recur as [Ca\textsuperscript{2+}] oscillations within localized regions.

ACh-Induced [Ca\textsuperscript{2+}] Oscillations Blocked by Depletion of SR Ca\textsuperscript{2+} Stores in Airway Smooth Muscle

- 1 μM ACh
- 5 mM Caffeine
Intracellular Ca\textsuperscript{2+} Regulation in Airway Smooth Muscle

**Key**
- Ca\textsuperscript{2+} stores in sarcoplasmic reticulum (SR) must be maintained
- SR depletion triggers store-operated Ca\textsuperscript{2+} entry – SOCE – via transient receptor protein channel TRPC (transient receptor protein channel)
- Triggering of SOCE involves translocation and aggregation of STIM1 (stromal interaction molecule) and interaction with ORAI1

**Store-Operated Ca\textsuperscript{2+} Entry (SOCE)**

**Cytokines Decrease SERCA2 Expression and Slow SR Ca\textsuperscript{2+} Reuptake in Airway Smooth Muscle**

**Store-Operated Ca\textsuperscript{2+} Entry (SOCE) in Airway Smooth Muscle Cells**

SOCE triggered by depletion of SR Ca\textsuperscript{2+} stores regardless of underlying cause
Cytokines Increase Store-Operated Ca\(^{2+}\) Entry in Airway Smooth Muscle

TRPC3 Knockdown Blunts TNF\(\alpha\)-Induced Increase in Store-Operated Ca\(^{2+}\) Entry in Airway Smooth Muscle

TNF\(\alpha\) Increases Expression of TRPC3 in Airway Smooth Muscle

Aggregation of STIM1 Following SR Ca\(^{2+}\) Depletion
STIM1 siRNA Knockdown Reduces Store-Operated Ca^{2+} Entry in Airway Smooth Muscle

Mitochondrial Ca^{2+} Regulation and SR Ca^{2+} Repletion

Mitochondria are juxtaposed to plasma membrane and SR
Mitochondria contain Ca^{2+} channels – Ca^{2+} uniporter, Na+/Ca^{2+} exchanger and Ca^{2+}/H^{+} exchanger
Mitochondrial ATP production is Ca^{2+} dependent and essential for SR Ca^{2+} reuptake (i.e., SERCA)

STIM1 Aggregates Towards TRPC3 Following SR Ca^{2+} Depletion

Cy3-tagged STIM1 (donor fluorophore) and Cy5-tagged TRPC3 (acceptor fluorophore). Acceptor photobleaching increases FRET efficiency when molecules are in close proximity

Proximity of SR, Mitochondria and Plasma Membrane Is Important in the Regulation of Intracellular Ca^{2+}
Mitochondria Move Away from Plasma Membrane during Agonist Stimulation

Mitochondria Move Toward SR during Agonist Stimulation and with SOCE

Mitochondria and SR are Functionally Coupled

Dynamic Coupling of Mitochondria and SR is Mediated by MFN2
**“Hot Spot” Theory for Mitochondrial Ca\(^{2+}\) Regulation**

**Cytosolic and Mitochondrial Ca\(^{2+}\) Responses are Coupled in Airway Smooth Muscle Cells**

**Inflammatory Cytokines Reduce MFN2 Expression in Airway Smooth Muscle**

**Inflammatory Cytokines Uncouple Cytosolic and Mitochondrial Ca\(^{2+}\) Responses in Airway Smooth Muscle**
**Inflammatory Cytokines Trigger SR-Mitochondrial Uncoupling in Airway Smooth Muscle**

![Image showing mitochondrial and SR localization](image1)

**Mitochondrial Network Fusion/Fission**

![Diagram showing mitochondrial fusion and fission](image2)

**Inflammatory Cytokines Trigger Mitochondrial Fragmentation in Airway Smooth Muscle**

![Graph showing 24 h exposure](image3)

**Inflammatory Cytokines Trigger Mitochondrial Fragmentation in Airway Smooth Muscle**

![Graph showing protein expression](image4)
Role of DRP1 (Fusion) and MFN2 (Fission) in Mitochondrial Dynamics in Airway Smooth Muscle

Effect of Inflammatory Cytokines on Excitation-Energy Coupling in Airway Smooth Muscle

Excitation-Energy Coupling in Airway Smooth Muscle

Inflammatory Cytokines Increase Force Generation in Airway Smooth Muscle
Estimating ATP Consumption Rate

ATP Consumption Increases with Force Generation in Airway Smooth Muscle

Inflammatory Cytokines Increase Tension Cost in Airway Smooth Muscle

Inflammatory Cytokines Increase Mitochondrial Respiration in Airway Smooth Muscle
Inflammatory Cytokines Increase Mitochondrial Respiration in Airway Smooth Muscle

Mitochondrial Fragmentation Leads to Mitogenesis in Airway Smooth Muscle

Inflammatory Cytokines Increase Reactive Oxidant Species (ROS) Generation in Airway Smooth Muscle

Excitation-Energy Coupling in Airway Smooth Muscle
Inflammatory Cytokines Trigger and Unfolded Protein Response (ER/SR Stress) in Airway Smooth Muscle

- Inflammation/ROS
- SR(ER) Stress
- PERK
- α-ATF6
- Splicing of XBP1 mRNA
- XBP1 protein
- SR stress target genes
- Chaperones, MFN2

Inflammatory Cytokines Trigger ER/SR and Mitochondrial Stress in Airway Smooth Muscle

- PERK Protein Expression (Relative to GAPDH)
- Control
- IL-13

- Spliced XBP1 mRNA Expression (Relative to GAPDH)
- Control
- IL-13

“There is no such thing as a new idea. It is impossible. We simply take a lot of old ideas and put them into a sort of mental kaleidoscope. We give them a turn and they make new and curious combinations.”

Mark Twain

Cellular Imaging and Physiology Laboratory
Department of Physiology & Biomedical Engineering

Research supported by grants from the NIH
HL96750, HL74309, HL37680, GM56686, & AR51173