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Mechanosensitive Modulation of Cytoskeleton and Signal Transduction in Airway System

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Airway smooth muscle cells function in a mechanically active environment, and constantly undergo shortening and lengthening during lung expiration and inspiration. Transpulmonary pressure tenses the elastic and connective tissues of lung parenchyma, which creates the elastic recoil load on airway smooth muscle cells. Mechanical strain (deformation) of the airway smooth muscle cell results from the balance of stress generated by a smooth muscle cell against the stress imposed by the elastic load. It is generally recognized that airway smooth muscle hyperresponsiveness and weakening of elastic recoil load are important causes of obstructive airway disease such as asthma. However, the feedback modulation of airway smooth muscle responsiveness by mechanical strain imposed by the elastic recoil load is not fully understood at the cellular and molecular level. Understanding these molecular mechanisms may offer new targets of pharmacological intervention in obstructive airway diseases such as asthma.

Our central hypothesis is that mechanical strain induces an increase in stress-bearing elements in an airway smooth muscle cell by crossbridge activation, cytoskeletal reorganization, gene expression, and protein expression. Crossbridge activation controls crossbridge attachment and cycling that produce force and shortening. Contractile and cytoskeletal filament organization controls the transmission of force from crossbridges to the extracellular matrix. The amount of contractile and cytoskeletal filaments controls the available number of stress-bearing elements in a cell. This increase in stress-bearing elements in turn allows the cell to bear more stress at a given strain, thereby minimizing the mechanical strain of the cell induced by the external elastic load.

Phosphatidylinositol turnover is a major signal transduction mechanism in airway smooth muscle cells. Ca^{2+}, calmodulin-dependent phosphorylation of the 20,000 dalton myosin light chain is the central regulatory mechanism of

contractile protein activation in smooth muscle. Findings from our laboratory indicate that mechanical strain modulates receptor-mediated phosphatidylinositol turnover, intracellular [Ca^{2+}], and myosin light chain phosphorylation, suggesting that signal transduction is the primary target of mechanosensitive modulation. Specifically, we found that mechanical strain modulates maximal receptor-mediated phosphatidylinositol turnover and myosin light chain phosphorylation without affecting affinity of agonist-receptor interaction. Fluoroaluminate activates trimeric G-proteins directly, thus bypassing receptor activation. We found that mechanical strain also modulates maximum phosphatidylinositol turnover induced by fluoroaluminate. These findings suggest that mechanical strain regulates the number of functional G-proteins and/or phospholipase C enzymes in the airway smooth muscle cell membrane possibly by membrane trafficking and/or protein translocation. It has been proposed that mechanical stress-dependent conformational change of the focal adhesion complex may be sufficient to regulate a whole array of protein kinase pathways, thereby regulating cell activation. On the contrary, we found that receptor-mediated intracellular [Ca^{2+}] correlated linearly with muscle length during isotonic shortening independent of external load ranging from 20% to 80% isometric force. These results indicate that mechanical strain, but not stress, is the primary mechanical signal for shortening-induced attenuation of intracellular [Ca^{2+}] in airway smooth muscle. Therefore, global shortening of a smooth muscle cell is necessary to modulate cell activation. This finding suggests that integrin receptor clustering and/or membrane trafficking may be the underlying mechanism.

Actin is essential for the motility of both non-muscle and muscle cells. In non-muscle cells, dynamic actin polymerization and depolymerization are the basic mechanisms of cell motility. In contrast, actin filaments are stable in skeletal muscle. Findings from our laboratory indicate that actin filaments are dynamic in airway smooth muscle cells. Specifically, we found that cytochalasins B and D attenuated contraction and induced changes in cell morphology as revealed by confocal microscopy. Furthermore, cytochalasin-induced disruption of actin filaments led to attenuation of intracellular [Ca^{2+}] and myosin light chain phosphorylation. However, receptor-mediated myosin light chain phosphorylation remained length-dependent. These results suggest that integrity of actin filaments is essential for signal transduction in airway smooth muscle cells. Furthermore, actin filaments involved in contraction and mechanosensitive modulation may be differentiated by their different sensitivities to cytochalasin.

Metavinculin and vinculin are dense-plaque proteins for the anchoring of

actin filaments in smooth muscle cells. Recently, we investigated the roles of mechanical strain and muscarinic receptor activation in modulating the association of metavinculin and vinculin with actin cytoskeleton in airway smooth muscle. Cytoskeletal fractions were extracted by ultracentrifugation. Vinculin and actin were measured by SDS-polyacrylamide gel electrophoresis and Western blotting. In unstimulated tissues, cytoskeletal metavinculin/actin ratio decreased slightly with muscle length. Muscarinic receptor activation stimulated an ~3-fold increase in the cytoskeletal metavinculin/actin ratio at optimal length. Furthermore, the cytoskeletal metavinculin/actin ratio in carbachol-activated tissues exhibited a length-dependence similar to the length-dependence of active force. Ca^{2+}-depletion with EGTA abolished the length-dependence of the cytoskeletal metavinculin/actin ratio. Changes in vinculin followed changes in metavinculin in these experiments. These results suggest that mechanical and receptor activation induce the recruitment of metavinculin and vinculin to the actin cytoskeleton. Therefore, the entire cytoskeletal structure appears to be highly dynamic in airway smooth muscle cells. Understanding the molecular mechanisms of cytoskeletal dynamics in airway smooth muscle cells may offer new pharmacological targets for controlling airway diameter in obstructive airway diseases such as asthma.