Jordan University of Science and Technology

Hypercontractility of Intestinal Longitudinal Smooth Muscle Induced by Cytokines Is Mediated by the Nuclear FactorkB/ AMP-Activated Kinase/Myosin Light Chain Kinase

Authors: Ancy D. Nalli, Divya P. Kumar, Sunila Mahavadi, Othman Al-Shboul, Reem Alkahtani,John F. Kuemmerle, John R. Grider, and Karnam S. Murthy

Abstract: Recent studies have identified AMP-activated kinase (AMPK) as a target ofCa21/calmodulin-dependent kinase kinase (CaMKKb) and a negative regulator of myosin light-chain (MLC) kinase (MLCK). The present study examined whether a change in expression or activity of AMPK is responsible for hypercontractility of intestinal longitudinal muscle during inflammation or in response to proinflammatory cytokines. In mouse colonic longitudinal muscle cells, acetylcholine (ACh) stimulated AMPK and MLCK phosphorylation and activity and induced MLC20 phosphorylation and muscle contraction. Blockade of CaMKKb with STO609 (7-oxo-7H-benzimidazo[2,1-a]benz[de]isoquinoline-3- carboxylic acid acetate) inhibited AMPK and MLCK phosphorylation and augmented MLCK activity, MLC20 phosphorylation, and smooth muscle cell contraction. In muscle cells isolated from the colon of TNBS (2,4,6-trinitrobenzenesulfonic acid)- treated mice or from strips treated with interleukin-1b or tumor necrosis factor-a, nuclear factor kB was activated as indicated by an increase in p65 phosphorylation and IkBa degradation, and AMPK was phosphorylated at a cAMP-dependent protein kinase (PKA)?specific site (Ser485) that is distinct from the stimulatory CaMKKb site (Thr172), resulting in attenuation of ACh-stimulated AMPK activity and augmentation of MLCK activity and muscle cell contraction. Inhibition of nuclear factor-kB activity with MG-132 (carbobenzoxy-L-leucyl-L-leucyl- L-leucinal Z-LLL-CHO) or PKA activity with myristoylated PKA inhibitor 14-22 amide blocked phosphorylation of AMPK at Ser485 and restored MLCK activity and muscle cell contraction to control levels. The results imply that PKA released from IkBa complex phosphorylated AMPK at a PKA-specific site and inhibited its activity, thereby relieving the inhibitory effect of AMPK on MLCK and increasing MLCK activity and muscle cell contraction. We conclude that hypercontractility of intestinal longitudinal muscle induced by