S 18-4

NEGATIVE REGULATION OF MYOSIN PHOSPHATASE BY Ca^{2+} IN VASCULAR SMOOTH MUSCLE: INVOLVEMENT OF THE NOVEL RHO REGULATOR PHOSPHOINOSITIDE 3-KINASE CLASS II ALFA ISOFORM

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Calcium ion is a primary determinant of phosphorylation level of the 20-kDa myosin light chain (MLC) and tension in smooth muscle. Phosphorylation of MLC is regulated by both myosin light chain kinase (MLCK) and myosin phosphatase (MLCP). It is established that an increase in the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) activates the calmodulin-dependent enzyme MLCK and induces an increase in MLC phosphorylation. We unveiled the Ca^{2+}-induced negative regulation of MLCP in vascular smooth muscle, indicating that Ca^{2+} induces stimulation of MLC phosphorylation by both MLCK activation and MLCP inhibition; an increase in the $[Ca^{2+}]_i$ induced by membrane depolarization and ionomycin brought about the activation of Rho, which is a molecular switch to inhibit MLCP through Rho kinase- dependent phosphorylation of the MLCP-regulatory subunit MYPT1. Consistently, membrane depolarization induced several fold stimulation of phosphorylation of MYPT1 and also the MLCP inhibitor protein CPI-17 in a Rho kinase inhibitor-sensitive manner. The expression of a dominant negative Rho mutant inhibited MYPT1 phosphorylation, MLC phosphorylation and contraction in isolated vascular smooth muscle cells. We observed that Rho activation induced by the receptor agonist noradrenaline is markedly inhibited by Ca^{2+} depletion, suggesting that Ca^{2+}-dependent Rho activation mechanism is considerably contributing to receptor agonist-induced Rho activation in vascular smooth muscle. We recently found that a phosphoinositide 3-kinase (PI3K) is required for Ca^{2+}-dependent Rho activation; the PI3K inhibitors wortmannin and LY294002 inhibited all of the Ca^{2+}-dependent Rho activation, MYPT1 and CPI-17 phosphorylation, MLCP inhibition, MLC phosphorylation and contraction. In isolated vascular smooth muscle cells, siRNA-mediated, selective downregulation of PI3K class II alfa isoform (PI3K-C2alpha) expression, but not the class I p110alpha, markedly inhibited Rho kinase-dependent MYPT1 phosphorylation, MLC phosphorylation and contraction. Noradrenaline as well as membrane depolarization stimulated the activity of PI3K-C2alpha, but not p110alpha, in a Ca^{2+}-dependent manner. Thus, these observations indicate the novel role of PI3K-C2alpha in Ca^{2+}-dependent Rho activation and consequent MLCP inhibition.

Key Words: smooth muscle, myosin phosphatase, Rho, calcium

S 19-1

MODULATION OF NEURONAL BK_{Ca} CHANNELS BY INTERACTING PROTEINS AND SYNTHETIC MOLECULES

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As a molecular integrator of biochemical and electrical signals, large-conductance calcium activated potassium channels (BK_{Ca} channels) play a key role in the control of neuro-excitability. It is known that the functional activities of the channels as well as their expression are regulated by numerous signaling pathways and suggested that the channels serve as a component of large protein complexes by interacting with various cellular proteins. In order to identify the partners interacting with neuronal BK_{Ca} channels, we launched large-scale screenings using molecular biological, biochemical and proteomic tools. I will discuss those proteins specifically interact with BK_{Ca} channels via their cytoplasmic domains, and affecting the expression and the function of the channels. Since they act as a feedback regulator controlling membrane voltages and intracellular Ca^{2+}, BK_{Ca} channels are also known as a valuable therapeutic target. From the screening of targeted chemical library, we were able to obtain a series of chemical compounds strongly potentiating the activity of neuronal BK_{Ca} channels. I will also present our recent results on the mechanism of allosteric modulation by these novel activators.