**MARCKS regulates vascular contractility**

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Myristoylated alanine-rich C-Kinase (MARCKS) is expressed in vascular smooth muscle cells (VSMCs), but its function is unclear. The present work investigates the role of MARCKS in regulating vascular tone.

Experiments were carried out using freshly isolated VSMCs and tissue lysates from rat and mice mesenteric arteries. Expression and interactions between MARCKS, voltage-dependent Ca2+ channels (VDCCs), and PIP2, were studied using western blot, immunocytochemical, co-immunoprecipitation, and dot-blot methods. The cell-permeable MARCKS inhibitor, MANS peptide, and MARCKS knockdown were examined on contractility using isometric tension recordings. The result of MANS peptide on VDCC activity was measured using whole-cell patch clamp techniques.

MARCKS was expressed in mouse and rat mesenteric arteries, predominantly at the plasma membrane. MANS peptide evoked concentration-dependent increases in vascular contractility, which were abolished by VDCC blockers. Knockdown of MARCKS significantly reduced contractions evoked by MANS peptide and methoxamine, an α1-adrenoceptor agonist. In un-stimulated tissue, MARCKS interacted with the L-type VDCC protein, CaV1.2, and PIP2. MANS peptide released PIP2 from MARCKS and increased interactions between CaV1.2 and PIP2. MANS peptide induced an increase in the peak amplitude, and a negative shift in activation and inactivation of whole-cell VDCC currents. Depletion of PIP2, with wortmannin, abolished excitatory effects of MANS peptide on whole-cell VDCC activity.

This is the first study to demonstrate that MARCKS is involved in regulating vascular contractility. We propose that in un-stimulated vessels, MARCKS prevents opening of VDCCs by acting as a PIP2 buffer, but following MARCKS inhibition PIP2 is released leading to channel gating and contractility.

**Key words:** MARCKS; PIP2, VDCCs, Vascular Contractility