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Differential phosphorylation of LZ+/LZ- MYPT1 isoforms by PKGIα: implication for vascular reactivity

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Background

MLC phosphatase is a trimeric enzyme composed of a catalytic subunit, a 20-kDa subunit of unknown function, and a myosin targeting subunit (MYPT1). During NO stimulation, PKGIα mediated phosphorylation of MYPT1 increases MLC phosphatase activity, which produces a decrease in force. Further, alternative splicing of a 3' exon produces two MYPT1 isoforms, which differ by the presence or absence of a leucine zipper (LZ); a LZ+ MYPT1 isoform is required for PKGIα induced smooth muscle relaxation.

Results

To examine the influence of MYPT1 structure on the ability of PKGIα to phosphorylate the protein, we used two MYPT1 fragments, which differed only by the presence (MYPT1LZ+) or absence (MYPT1LZ-) of the LZ. Purified PKGIα phosphorylated MYPT1LZ+, but not MYPT1LZ-. Following phosphorylation, MYPT1LZ + predominantly existed as a di-phosphorylated protein, and mass spectrometry identified S^{668} and S^{695} as PKGIα-mediated phosphorylation sites. To examine the relative rates of S^{668} vs S^{695} MYPT1 phosphorylation, these residues were mutated to either A or D. The rates of D^{668} and A^{668} MYPT1 phosphorylation were similar and slow. The D^{695} MYPT1 mutant had the highest rate of phosphorylation, while the rate of phosphorylation of the A^{695} MYPT1 mutant was intermediate between that for the D^{695} MYPT1 and either A^{668} or D^{668} MYPT1.

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Conclusion

These results suggest that PKGIα-mediated phosphorylation of S^{695} is slower than S^{668}, and could suggest that PKGIα-mediated phosphorylation of S^{668} is physiologically significant for the regulation of MLC phosphatase activity. Further, MYPT1 structure has an important role in the regulation of vascular tone, and differential tissue expression of LZ+/LZ- MYPT1 isoforms contributes to the diversity in the sensitivity of smooth muscle to NO mediated vasodilatation.

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