BMC Pharmacology

Oral presentation

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The PKG1 specific phosphorylation substrate *cysteine-rich protein 2 knockout* decreases blood pressure and intimal hyperplasia

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from 4th International Conference of cGMP Generators, Effectors and Therapeutic Implications
Regensburg, Germany. 19–21 June 2009

Published: 11 August 2009

BMC Pharmacology 2009, **9**(Suppl 1):S33 doi:10.1186/1471-2210-9-S1-S33

This abstract is available from: http://www.biomedcentral.com/1471-2210/9/S1/S33

Introduction

The cysteine-rich protein 2 has previously been suggested as a novel target of PKG1. Cysteine-rich proteins (CRP) are evolutionarily conserved proteins that define a subset of zinc-binding LIM domain proteins. This family of LIM domain proteins originally included three members (CRP1, CRP2/SLIM, CRP3/MLP). Previously, a new member of the CRP family was identified through a yeast two hybrid screen using PKG1 as bait. This protein was initially named CRP2. Subsequently, based on structural and sequence similarities, and because it is the product of a distinct gene and not an ortholog of CRP2/smLIM this new CRP2 was grouped into the CRP1, CRP2/SLIM, CRP3/MLP subset of LIM domain proteins and is also referred to as CRP4. CRP4 is expressed in laminas I and II of the mouse spinal cord and is colocalized with PKG1 and the dorsal root ganglion (DRG) marker proteins calcitonin gene-related peptide, isolectin B4 and peripherin. CRP4 is phosphorylated in a cGMP-dependent manner, and its expression increases in the spinal cord and in DRGs after noxious stimulation of a hindpaw. CRP4$^{-/-}$ mice show increased nociceptive behavior in models of inflammatory hyperalgesia compared to wild-type mice. Intrathecal administration of cGMP analogs increases the nociceptive behavior in wild-type but not in CRP4$^{-/-}$ mice, indicating that the presence of CRP4 is important for cGMP-mediated nociception. CRP4 has been suggested as a new effector of PKG1-mediated spinal nociceptive processing and point to an inhibitory role of CRP2 in the generation of inflammatory pain.

Results

Here we show, that CRP4, apart from DRG neurons, is predominantly expressed in vascular smooth muscle cells. CRP4 is phosphorylated by PKG1 but not PKA in blood vessels. Cyclic GMP/PKG-mediated relaxation is enhanced in CRP4$^{-/-}$ small arteries, whereas cAMP-mediated vasorelaxation is not affected. Consistently, long-term radiotelemetric recordings reveal a decrease of mean arterial pressure by 10 mmHg and an enhanced NO/cGMP signaling in CRP4$^{-/-}$ mice. Western blotting analysis show increased expression of soluble guanylyl cyclase (sGC) in CRP4$^{-/-}$ vascular smooth muscle, whereas the expression of PKG and its effectors is not altered. Two-dimensional difference gel electrophoresis of the aortic proteome reveal caldesmon, an actin and tropomyosin binding protein, as well as vimentin and zyxin to be up-regulated whereas vinculin and smooth muscle protein SM22 are down-regulated at their expression level. An observed increase of adhesion of CRP4$^{-/-}$ aortic cells under basal and cGMP stimulated conditions may be a consequence of up-regulated cytoskeletal proteins such as cald-

esmon and zyxin. In agreement with increased adhesion of vascular smooth muscle cells, magnet resonance imaging and histological stainings demonstrate a decreased vessel wall thickness after injury of the carotid artery in CRP4$^{-/-}$ mice.

Conclusion

Our findings elucidate CRP4 as new and specific *in vivo* effector of the vascular NO/cGMP/PKG signaling and provide some new insights in the functional relevance of this protein.