BMC Pharmacology

Oral presentation

Regulation of smooth muscle specific gene expression by PKG and mechanisms regulating PKG expression in smooth muscle cells

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from 2nd International Conference of cGMP Generators, Effectors and Therapeutic Implications
Potsdam, Germany, 10–12 June, 2005

Published: 16 June 2005

BMC Pharmacology 2005, **5**(Suppl 1):S14 doi:10.1186/1471-2210-5-S1-S14

Cyclic GMP-dependent protein kinase I (PKG) is highly expressed in smooth muscle cells (SMC) and mediates the effects of nitric oxide (NO) on smooth muscle relaxation and SMC-specific gene expression. To understand the mechanisms by which PKG stimulates SMC-specific gene expression, we examined the effects of PKG over-expression in passaged rat aortic SMC that express low levels of PKG and SMC-specific genes. PKG enhances serum-response factor (SRF) and myocardin (MY)- induced reporter gene expression in SMC. These effects were not dependent on induction of either SRF or MY. The Ternary Complex Element transcription factor, Elk-1, is known to inhibit SRF-MY induced SMC gene expression when phosphorylated in response to platelet derived growth factor (PDGF). PKG inhibited Elk-1 repression of SRF-MY gene transcription by stimulating post-translational modification of phospho-Elk-1 via the small ubiquitin-like modifier (SUMO). The mechanism of PKG-induced sumoylation of Elk-1 may be dependent on the phosphorylation of SENP-1 (sumo-specific protease 1) at ser-125. These results suggest that PKG regulates SRF-MY gene expression through de-repression of Elk-1 on SMC-specific promoters.

PKG expression is highly variable in SMC and recent studies have shown that inflammatory cytokines (TNF and IL-1) inhibit PKG mRNA and protein expression in primary cultures of vascular SMC. In order to gain further insight into the mechanism of PKG repression by inflammatory mediators, we examined the role of cGMP itself in regulating PKG protein expression. Incubation of SMC with phosphodiesterase-resistant cGMP analogs decreased PKG-I protein levels in a ubiquitin-dependent manner. Overexpression of soluble guanylyl cyclase alpha and beta subunits and treatment with long-acting NO donors also down-regulated PKG protein expression. Furthermore, treatment of Cos7 cells with 8-Br-cGMP (which normally do not express PKG) that over-express PKG resulted in ubiquitination and down-regulation of PKG expression. Inhibition of proteosome dependent proteolysis with MG132 increased both PKG ubiquitination and PKG protein levels in Cos7 cells. Down-regulation of PKG protein expression and ubiquitination by cGMP was also blocked with DT-2, a PKG-I specific inhibitor, suggesting that autophosphorylation of PKG may important in PKG ubiquitination. These results suggest that the levels of PKG-I in cells are carefully controlled by elevations in cGMP and excessive cGMP production down-regulates PKG protein expression through the ubiquitin-proteosome pathway. These results also provide a possible role for autophosphorylation of PKG-I in controlling enzyme levels.

Taken together, these studies suggest that PKG plays an important role in regulating SMC growth and phenotype. Signaling through the NO-cGMP pathway enhances SRF-MY dependent gene expression that leads to increased expression of SMC contractile proteins. A decrease in PKG expression in SMC under normal physiological conditions of inflammation may be dependent on cytokine-induced elevation of cGMP in the cells, which leads to proteosome dependent degradation of PKG. Down-regulation of PKG expression may be an important adaptation of SMC in response to inflammation thereby allowing SMC to undergo phenotypic changes necessary for normal vessel growth and repair.