BMC Pharmacology

Oral presentation

Phosphorylation of cAMP hydrolyzing PDE (PDE3A) by cGMP-dependent protein kinase (PKG) in human platelets

Sergei D Rybalkin*, Irina G Rybalkina and Joseph A Beavo

Address: Department of Pharmacology, Box 357280, University of Washington, Seattle, WA 98195, USA

Email: Sergei D Rybalkin* - sergeir@u.washington.edu

* Corresponding author

from 3rd International Conference on cGMP Generators, Effectors and Therapeutic Implications
Dresden, Germany. 15–17 June 2007

Published: 25 July 2007

BMC Pharmacology 2007, **7**(Suppl 1):S29 doi:10.1186/1471-2210-7-S1-S29

This abstract is available from: http://www.biomedcentral.com/1471-2210/7/S1/S29

Endogenous inhibitory mediators such as prostacyclin and nitric oxide keep platelet activation under tight control through the corresponding cGMP and cAMP signaling pathways. Intracellular levels of cAMP and cGMP are regulated by cyclic-nucleotide phosphodiesterases (PDEs). PDE3A (also known as cGMP-inhibited cAMP PDE) and PDE5 (cGMP specific PDE) are major cAMP-hydrolyzing and cGMP hydrolyzing PDEs expressed in platelets. Platelet PDE3A has been found to be phosphorylated and activated by PKA [1] and PDE5 by PKG [2]. Here we report that in human platelets PKG can also phosphorylate PDE3A, providing an additional cross-talk between cGMP and cAMP signaling.

In non-treated human platelets PDE3A has been identified as a band with an apparent molecular weight of 110 kDa. Treatment of human platelets with SNP resulted in a time dependent mobility shift in SDS-PAGE. ODQ completely blocked the change in mobility for PDE3A as well as PKG induced phosphorylation of PDE5. Two PKG activators (8-Br-cGMP and 8-pCPT-cGMP) produced similar changes in PDE3A mobility, which were also parallel with changes in PDE5 phosphorylation. The PKG inhibitor (Rp-8-Br-PET-cGMPS) prevented phosphorylation of both PDE3A and PDE5 induced by 8-pCPT-cGMP.

In vitro phosphorylation of human platelet PDE3A by the addition of exogenous PKG produced the same shift in mobility of PDE3A in SDS-PADE as all experiments performed on intact platelets. Phosphorylation of PDE3A by PKA in vitro or in forskolin-treated platelets also resulted in changes in its mobility.

In platelets application of cGMP-elevating agents leads not only to higher intracellular cGMP concentration, but also to higher cAMP concentration through direct inhibition of PDE3A by cGMP. Our data provide evidence that PKG is able to control the duration of both cAMP and cGMP signalling by simultaneous phosphorylation of both PDE3A and PDE5 in human platelets.

References

1. Macphee CH, Reifsnyder DH, Moore TA, Lerea KM, Beavo JA: **Phosphorylation results in activation of a cAMP phosphodiesterase in human platelets.** *J Biol Chem* 1988, **263:**10353-10358.
2. Mullershausen F, Russwurm M, Thompson WJ, Liu L, Koesling D, Friebe A: **Rapid nitric oxide-induced desensitization of the cGMP response is caused by increased activity of phosphodiesterase type 5 paralleled by phosphorylation of the enzyme.** *J Cell Biol* 2001, **155:**271-278.