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Poster presentation

IRAG is involved in NO/cGMP-mediated inhibition of platelet function

Melanie Antl, Katja Sigl*, Christina Eigelsperger, Matthias Werner, Franz Hofmann and Jens Schlossmann

Address: Institut für Pharmakologie und Toxikologie, Technische Universität München,80802 München, Germany

Email: Katja Sigl* - sigl@ipt.med.tu-muenchen.de

* Corresponding author

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The inositol 1,4,5-trisphosphate receptor-associated cGMP kinase substrate (IRAG) is highly expressed in platelets. It is assembled in a macrocomplex together with cGMP-dependent protein kinase Iβ (cGKIβ) and the inositol 1,4,5-trisphosphate receptor type I (IP_3RI). In response to cGKI activation, IRAG is phosphorylated in vivo. By mass spectrometric analysis of purified in vivo phosphorylated IRAG from cGMP-stimulated human platelets, Ser664 and Ser677 were identified as phosphorylated amino acids. Generation of phosphospecific antibodies confirmed these in vivo phosphorylation sites. To examine the role of IRAG in platelet function, we analysed the aggregation of platelets from an IRAG mouse mutant ($IRAG^{\Delta 12/\Delta 12}$). In this mutant, exon 12 of the IRAG gene was deleted and thereby the IRAG-IP_3RI interaction was abolished [1]. Interestingly, collagen-induced aggregation of $IRAG^{\Delta 12/\Delta 12}$ platelets was not inhibited by nitric oxide (NO) and the cGMP analogue 8-pCPT-cGMP in contrast to wild type platelets. The shape change was not affected, neither in mutant nor in wild type platelets. However, iloprost and the cAMP-analogue cBIMPS still inhibited aggregation and shape change in $IRAG^{\Delta 12/\Delta 12}$ and wild type platelets. Additionally, we analysed fibrinogen binding to thrombin-stimulated platelets. In wild type platelets, pre-treatment with NO and cGMP decreased fibrinogen binding by about 50%. In contrast, in $IRAG^{\Delta 12/\Delta 12}$ platelets the response to NO and cGMP was nearly absent. Preincubation with iloprost and cBIMPS clearly reduced agonist-induced fibrinogen binding to both, $IRAG^{\Delta 12/\Delta 12}$ and wild type platelets. These results suggest that signalling through IRAG/IP_3RI is essential for NO/cGMP-dependent inhibition of platelet aggregation and activation of the fibrinogen receptor

References

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