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Poster presentation

Characterization of an epitope-tagged guanylyl cyclase-A receptor

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Background

In some forms of arterial hypertension and as one of the earliest and pathognomonic events in cardiac hypertrophy and insufficiency, the cardiac synthesis and release of ANP and BNP is markedly enhanced, but the GC-A-mediated effects of the NPs are clearly diminished, indicating a receptor or postreceptor defect of GC-A. Thus, from a pathophysiological perspective, identifying the specific mechanisms involved in the downregulation of GC-A activity has important implications. Biochemical studies in transfected GC-A-overexpressing cells showed that phosphorylation of GC-A is essential for its activation process. In turn, inactivation of GC-A probably involves dephosphorylation [1]. NP-dependent activation of GC-A can be reduced not only by chronic exposure to NPs (homologous desensitization) but also by exposure to agents other than NPs (heterologous desensitization). It has been shown in cell culture systems that Angiotensin II and endothelin decrease the responsiveness of GC-A. This is probably mediated by a protein kinase C-induced dephosphorylation of GC-A. However, ultimately the precise posttranslational modifications leading to homologous vs. heterologous GC-A desensitization have not been fully characterized. The present study describes the functional characterization of an epitope-tagged GC-A receptor which might help to enrich the receptor for the characterization of putative posttranslational modifications.

Methods, results and conclusion

The FLAG (DYKDDDDK) epitope was positioned immediately after the cleavage site of the signal peptide by PCR-mediated mutagenesis (pCMV5-FLAG-GC-A) [2]. HEK cells were transfected with cDNAs for GC-A or GC-A tagged with the FLAG epitope.

To determine cellular cGMP responses, transfected cells on 24-well plates were pretreated with 0.1 mM 3-isobutyl-1-methylxanthine for 15 min and then stimulated with ANP during additional 10 min. Ethanol (70%, v/v) was used to terminate the reaction. Cyclic GMP in the cell extracts was quantitated by radioimmunoassay. ANP (1 pM – 100 nM) elicited concentration-dependent increases in cellular cGMP which were nearly identical in GC-A – and FLAG-GC-A – transfected HEK cells. Pretreatment of the cells with the protein kinase C activator PMA (200 nM, 30 min) dramatically reduced these responses.

Additionally, fluorescence resonance energy transfer (FRET) with a new sensor for cGMP [3] was used to monitor the temporal and spatial resolution of cGMP elevations. Both, the kinetics, duration and extent of cGMP formation in response to ANP were similar in GC-A and FLAG-GC-A – transfected HEK cells.

We conclude that addition of the FLAG-epitope does not interfere with the ability of the receptor to bind or be activated by ANP. In future studies we will apply this tool to

enrich the protein by immunoprecipitation and characterize putative modifications by mass spectrometry.

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