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Poster presentation

A splice variant of the guanylyl cyclase-A receptor interferes with atrial natriuretic peptide (ANP) signaling

Michael Hartmann*[1], Katharina Völker[1], Birgit Gaßner[1], Juliane Schröter[1], Beatrice Dankworth[1], Thomas Müller[2], Viacheslav O Nikolaev[3] and Michaela Kuhn[1]

Address: [1]Institute of Physiology, University of Würzburg, Germany, [2]Department of Molecular Plant Physiology and Biophysics, University of Würzburg, Germany and [3]Institute of Pharmacology and Toxicology, University of Wurzburg, Germany

Email: Michael Hartmann* - m.hartmann@uni-wuerzburg.de

* Corresponding author

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Background

Activation of the homodimeric transmembrane guanylyl cyclase-A (GC-A) receptor upon binding of its extracellular ligands, atrial (ANP) and B-type (BNP) natriuretic peptides, leads to cyclic GMP formation in many types of cells. This NP/GC-A pathway has a critical role in the endocrine regulation of arterial blood pressure and volume and in the local counter-regulation of cardiac hypertrophy and fibrosis. Alterations of this system result in arterial hypertension, hypervolemia and cardiac hypertrophy. Many studies have shown that exposure of GC-A to high concentrations of ANP/BNP or to growth hormones such as angiotensin II (Ang II) or endothelin provokes homologous versus heterologous desensitization of the receptor [1]. However, the mechanisms accounting for this loss of function of GC-A *in vivo* are largely unknown. In the present study we identified and characterized a novel isoform of GC-A (GC-A$\Delta_{Lys}{}^{314}$-$_{Gln}{}^{330}$) with unique structural properties. Our data reveal that this splice variant functions as dominant negative isoform and suggest that increased alternative splicing of GC-A may contribute to homologous or heterologous desensitization of the NP/GC-A system.

Methods and results

Reverse transcription-PCR analyses revealed a novel isoform of GC-A mRNA which is expressed in all studied murine tissues such as heart, kidney and lung, although at a ~10-fold lower level compared to the wild-type receptor. The isoform results from the deletion of a 51-bp sequence in exon 4, generated by splicing a cryptic donor site to the normal acceptor site. This alternative splicing is predicted to delete 17 amino acids ($\Delta_{Lys}{}^{314}$-$_{Gln}{}^{330}$) in the membrane-distal part of the extracellular ligand-binding domain of GC-A.

The deletion is not located directly within the ligand-binding site of GC-A (which is within the membrane-proximal part of the extracellular domain) and therefore a direct influence on ANP and/or BNP binding seemed unlikely. However, molecular modeling of the extracellular domain of the splice variant indicated that subsequent alterations in the protein structure could interfere with NP binding or signaling. Indeed, our functional studies with transiently transfected HEK-293 cells demonstrated that binding of ANP and ANP-induced cyclic GMP formation by GC-A$\Delta_{Lys}{}^{314}$-$_{Gln}{}^{330}$ were totally abolished. Furthermore, cotransfection studies showed that this GC-A variant forms heterodimers with the wild-type receptor and inhibits ligand-inducible cGMP generation, acting as a dominant negative isoform.

To elucidate whether increased alternative splicing of GC-A could participate in the negative regulation of the NP/

GC-A system by Ang II *in vivo* we examined the expression and activity of GC-A in lungs from control mice, and from mice treated with Ang II at a dose of 300 ng/kg/min during 7 days. Ang II – treated mice showed a markedly enhanced pulmonary mRNA expression of spliced GC-A which was concomitant to diminished GC-A/cGMP responses to ANP.

Our ongoing in vitro studies are directed to investigate whether alternative splicing of GC-A does occur in all cells expressing the receptor, or only in specific cell types. To address this question we use real-time RT-PCR to determine the ratio of wild-type versus spliced GC-A mRNA expression in different primary cultured murine cells. First results demonstrate that the splice variant is expressed in cultured microvascular lung endothelial cells (MLEC). Treatment of MLEC with ANP (100 nM during 7 h) enhanced the mRNA expression of the spliced GC-A isoform, suggesting that alternative splicing could be involved not only in heterologous (Ang II-induced) but also in homologous (NP-induced) inactivation of GC-A.

Conclusion

We conclude that alternative splicing can regulate endogenous NP/GC-A signaling. Our observations *in vivo*/*in vitro* indicate that Angiotensin II – and ANP-induced alternative splicing of GC-A may represent a novel mechanism for reducing the sensitivity of the receptor to ANP and BNP.

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