BMC Pharmacology

Poster presentation

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Determination of new phosphorylation sites within natriuretic peptide receptors using mass spectrometric methods

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from 4th International Conference of cGMP Generators, Effectors and Therapeutic Implications
Regensburg, Germany. 19–21 June 2009

Published: 11 August 2009

BMC Pharmacology 2009, **9**(Suppl 1):P75 doi:10.1186/1471-2210-9-S1-P75

This abstract is available from: http://www.biomedcentral.com/1471-2210/9/S1/P75

Background

While it has long been know that phosphorylation of the kinase homology domain is a key regulatory mechanism of natriuretic peptide receptors, no biochemical proof for individual phosphorylation sites has been reported. Here, we describe biochemical verification of previously identified phosphorylation sites in both natriuretic peptide receptor A (NPR-A) and natriuretic peptide receptor B (NPR-B) as well as the identification of novel phosphorylation sites in both receptors.

Methods and results

Natriuretic peptide receptors were immunoprecipitated and separated by gel electrophoresis. Isolated receptors were digested in the gel by tryptic digestion and individual phosphopeptides were detected with a LTQ-Orbitrap XL hybrid Fourier transform mass spectrometer. Tandem mass spectrometry of the tryptic peptide mixture revealed phosphorylation at all of the previously identified phosphorylation sites within NPR-A and NPR-B. Evidence of additional uncharacterized phosphorylation sites was also obtained. Functional consequences of these individual phosphorylation sites were determined through the use of alanine and glutamate point mutations, which mimic the dephosphorylated and phosphorylated receptor, respectively.

Conclusion

Using mass spectrometric techniques, we have successfully verified known natriuretic peptide receptor phosphorylation sites as well as identified additional novel phosphorylation sites. Possible functional consequences of these putative sites on receptor activation as determined by alanine and glutamate substitutions will be discussed.