

POSTER PRESENTATION

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Influence of PKG on insulin signalling and GSK3 phosphorylation in SH-SY5Y cells

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From 6th International Conference on cGMP: Generators, Effectors and Therapeutic Implications
Erfurt, Germany. 28-30 June 2013

Background

Extracellular Amyloid- β (A β) plaques and intracellular neuro-fibrillary tangles (NFTs) of hyperphosphorylated Tau (τ) protein are considered to be the hallmarks of Alzheimer's disease (AD) [1]. A β is secreted due to the sequential cleavage of the amyloid β precursor protein (APP) by β - and γ - secretases (β cleavage) [1], whereas the intracellular signalling protein glycogen synthase kinase 3 (GSK3) has been implicated to cause τ - hyperphosphorylation leading to the formation of NFTs [2].

It has been shown earlier that the cleavage of APP by α - and γ - secretases (α - cleavage) is enhanced by insulin through the PI3K- Akt pathway [3]. GSK3 is a further downstream component of this pathway which has been shown to induce τ phosphorylation. Inhibition of GSK3 has also been shown to increase lysosomal biogenesis leading to autophagic degradation of APP [4].

Materials and methods

For the purposes of this study, human neuroblastoma (SH-SY5Y) cells were used. Cells expressing wtAPP, bovine cGMP dependent protein kinase 1- α (PKG1 α) and murine PKG2 were generated by lentiviral transduction and were stimulated with 200 μ M 8-pCPT-cGMP or/ and 1 μ M insulin for 15 min or 2 hrs. The cells were then lysed and the proteins analysed by Western Blotting.

Results

SH-SY5Y cells stably overexpressing APP, PKG1 α and PKG2 were used to analyze the crosstalk between the cGMP-PKG and insulin signalling cascades that was reported in brown adipose tissue [5]. As is known, upon insulin stimulation, the APP overexpressing cells showed a marked increase in the α -cleavage of APP with increased

secreted APP α levels (sAPP α). Analyzing the insulin pathway components in the cells overexpressing PKG (1 α or 2), a significant increase in phosphorylation of GSK3 was also seen when these cells were stimulated with cGMP, implying that PKG influences the downstream phosphorylation events in insulin signalling. While, PKG1 α overexpressing cells also showed a marked reduction in intracellular holoAPP levels with consequent reduction in extracellular sAPP α levels as well.

Conclusion

Our results suggest a possible crosstalk between cGMP-PKG and insulin signalling cascades. PKG1 α and PKG2 enhanced GSK3 phosphorylation upon cGMP stimulation, while PKG1 α affected levels of intracellular holoAPP thus reducing extracellular sAPP α levels.

Influence of PKG on GSK3 phosphorylation renders it as a viable and valuable target for AD therapeutics following a two-pronged approach; to reduce secreted A β levels by enhancing lysosomal biogenesis and simultaneous τ hyperphosphorylation reduction.

Published: 29 August 2013

References

1. Guo Q, Wang Z, Li H, Wiese M, Zheng H: APP physiological and pathophysiological functions: insights from animal models. *Cell Res* 2012, **22**:78-89.
2. Hanger DP, Hughes K, Woodgett JR, Brion JP, Anderton BH: Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of airod helical filament epitopes and neuronal localisation of the kinase. *Neurosci Lett* 1992, **147**:58-62.
3. Adlerz L, Holback S, Multhaup G, Iverfeldt K: IGF-1-induced Processing of the myloid Precursor Protein family is Mediated by different signaling pathways. *J Biol Chem* 2007, **282**:10203-10209.
4. Parr C, Carzaniga R, Gentleman SM, Van Leuven F, Walter J, Sastre M: Glycogen synthase kinase 3 inhibition promotes lysosomal biogenesis and autophagic degradation of the amyloid- β precursor protein. *Mol Cell Biol* 2012, **32**:4410-4418.

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5. Haas B, Mayer P, Jennissen K, Scholz D, Berriel Diaz M, Bloch W, Herzig S, Fässler R, Pfeifer A: **Protein kinase G controls brown fat cell differentiation and mitochondrial biogenesis.** *Sci Signal* 2009, **2**:a78.

doi:10.1186/2050-6511-14-S1-P60

Cite this article as: Sanyal et al.: Influence of PKG on insulin signalling and GSK3 phosphorylation in SH-SY5Y cells. *BMC Pharmacology and Toxicology* 2013 **14**(Suppl 1):P60.

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