EP1THE0RESE KLIN IKES FARMACOLOGIAS KAI FARMAKOKINETIKES, INTERNATIONAL EDITION 11: 48 (1997)

Cross-Talk between Regulatory Systems: Phosphorylation of Steroid Hormone Receptors

Marija Krstic, Michael Garabedian, Natasha Pocuca and Keith Yamamoto

Key words: Phosphorylation, steroid hormone receptors

Division of Biochemistry and Molecular Biology, Institute of Biochemical and Life Sciences, University of Glasgow, Scotland

Accepted for publication: 15 April 1997

We have studied the role of phosphorylation in glucocorticoid receptor function using various experimental approaches involving both mammalian and yeast cells; in particular we studied how receptor function is modulated by phosphorylation, and the kinases involved in receptor phosphorylation. In the first part of our study we examined receptor functions affected by phosphorylation. The rat glucocorticoid receptor is competent for signal transduction and transcriptional regulation when expressed in *Saccharomyces cerevisiae*. We found that the receptor in yeast, as in mammalian cells, we phosphorylated in the absence and presence of hormone, and that additional receptor phosphorylation accompanied agonist binding. The receptor was phosphorylated on Ser and Thr residues, and two-dimensional tryptic and V8 phosphopeptide maps of receptor from yeast and mammalian cells were qualitatively similar; we showed directly that T171, S224, S232 and S246 were phosphorylated in yeast *in vivo*, as they are in animal cells. T171 and S246 were phosphorylated constitutively, whereas phosphorylation of S224 and S232 was increased in the presence of hormone agonists. In both yeast and animal cells, transcriptional enhancement was reduced by mutation of S224 and increased by mutation of S246. In contrast, transcriptional repression was unaffected by either mutation. We conclude that the glucocorticoid receptor phosphorylation affects selectively its transcriptional regulatory functions. In the second part, we investigated the kinases responsible for receptor phosphorylation. We found that cyclin-dependent kinase (CDK) and mi- togen-activated protein kinase (MAPK) phosphor- ylate the rat glucocorticoid receptor *in vivo]* MAP kinase phosphorylates receptor residues threonine 171 and serine 246, whereas cyclin-dependent kinase modifies serines 224 and 232. Mutations in these kinases have opposite effects on receptor transcriptional activity *in vivo*. Receptor dependent transcriptional enhancement is compromised in yeast strains deficient in the catalytic (p34 cdc28) or in certain regulatory subunits (cy- clins) of the cyclin dependent kinase and is potentiated in a strain of yeast devoid of the mammalian MAP kinase homologues FUS3 and KSS1. These findings indicate that the glucocorticoid receptor is a target for multiple kinases *in vivo*, which regulate either positively or negatively receptor transcriptional enhancement. We conclude that the receptor receives and integrates information from multiple regulatory inputs, including steroids, and at least two signaling pathways that modulate distinct protein kinases.

Acknowledgement

This work is supported by the Ministry of Energy and Technology of Greece