Investigation of the fundamental mechanisms underlying the ligand-selective regulation of gene expression by the glucocorticoid receptor

Ronacher K., Stubsrud E., Avenant C., Koubovec, D., Louw A. and Hapgood J.P.

Department of Biochemistry, University of Stellenbosch, Matieland 7602, Stellenbosch, South Africa

Endogenous glucocorticoids (GCs) maintain basal and stress-related homeostasis by modulating a large number of metabolic, cardiovascular, immune and behavioural functions. Synthetic GCs are widely used pharmacologically, for example in the treatment of inflammatory and autoimmune diseases. GCs mediate their effects via the glucocorticoid receptor (GR), a ligand-activated transcription factor, and member of the steroid receptor family of proteins. It is well-established that the affinity of a ligand for the GR does not correlate with potency. A central question thus remains: which of the multiple steps in the intracellular pathway of GC action play a role in determination of biological activity? Towards answering this question, we investigated whether ligand potency for transcriptional regulation correlates with the stability of the GR-ligand complex. Effects on both transactivation and transrepression of synthetic promoter-luciferase constructs were investigated with a range of different GR-ligands. In parallel, Western blot analysis of the GR protein was performed to determine the half-life of the receptor after ligand exposure. We also investigated whether a correlation exists between ligand potency and receptor phosphorylation, using phospho-serine specific antibodies to detect differences in the phosphorylation pattern with different GR-ligands. Finally, the results were also compared to the binding-affinities of the ligands for the GR, as determined by whole cell competitive binding assays. The results have important implications for drug design and provide insights into the mechanism of action of steroid receptors.