Expression and function of nuclear receptor coregulators in brain: understanding the cell-specific effects of glucocorticoids

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Chapter VII

SUMMARY

Based on the concept described by the French physiologist Claude Bernard (1813-1878) of 'le milieu interieur', Walter Cannon (1871-1945), a pioneering 20th century American physiologist, formulated the idea of homeostasis for living organisms. He introduced the term in the following context: "The coordinated physiological reactions which maintain most of the steady states in the body are so complex, and are so peculiar to the living organism, that it has been suggested that a specific designation for these states be employed - homeostasis" (1929). In most mammals, when homeostasis is threatened, such as in a situation of acute danger, a hormonal cascade initiating in the brain, known as the hypothalamus pituitary adrenal axis (HPA axis), is activated. As a result, blood glucocorticoid levels increase to support return to homeostatic set-point by enhancing e.g. neuroplasticity in the brain which underlies behavioural adaptation.

Currently, the raising awareness of the role of glucocorticoids in the onset of numerous (neuro)-pathologies constitutes the increasing necessity of understanding the mechanisms of action of glucocorticoids in bodily processes and brain functioning. Glucocorticoids mediate their effects by binding to intracellular receptors which act as transcription factors. A remarkable and yet unexplained phenomenon described more than two decades ago, is the cell-specific effects glucocorticoids bring about on gene expression in brain. For example, while glucocorticoids suppress corticotrophin-releasing hormone (CRH) synthesis in the hypothalamus, production of CRH in the central nucleus of the amygdala (CeA) is stimulated by increased hormone levels. Inasmuch as the neuroanatomical distribution of the corticosteroid receptors does not satisfactorily explain these effects, it is of interest to decipher the role of recently discovered coregulator proteins that modulate the direction and the magnitude of steroid receptor-driven transcription. Therefore, in the current thesis the expression and function of central coregulators was studied. In addition, a method that allows detection of coregulator recruitment by steroid receptors in brain tissue was developed.

In chapter 2, the expression levels of two functionally distinct corepressors, *i.e.* N-CoR and SMRT, were mapped in the rodent brain and pituitary. Clear differences in relative mRNA and protein expression levels were discerned in discrete brain areas critically involved in the regulation of the HPA axis such as the PVN. In hippocampus, N-CoR and SMRT were shown to colocalize in the nucleus although complexes containing exclusively N-CoR or SMRT were also detected. Unexpectedly, cytoplasmic N-CoR immunoreactivity was observed in pyramidal neurons of the frontal cortex and of hippocampus, suggesting, an additional role for this corepressor protein, apart form its function in nuclear receptor signalling. These findings strengthen the idea that coregulator proteins may underlie the cell-specific effect of glucocorticoids, particularly if the distinct differences in expression levels of these two functionally distinct corepressors are considered.

In chapter 3, based on the above mentioned uneven distribution in rodent brain of the corepressors and the previously described SRC1 expression levels, the effect of these coregulators on the transcriptional activity of GR was assessed by measuring an endogenous glucocorticoid target gene. In view of the model described in 1999 by Szapary and colleagues coactivators and corepressors were thought to mediate opposing effects on GR-driven transcription. This implies that corepressors were expected to induce a right-shift of the dose-response curve while coactivators should cause a left- shift. Unexpectedly, in the AtT20 cells, a model system for GR-mediated repression of the CRH gene, overexpression of the corepressors did not affect the glucocorticoid-induced repression of the human CRH gene via GR, but rather markedly impaired the cAMP-induced stimulation of the CRH gene. Interestingly, SRC1a overexpression increased both efficacy and potency of the glucocorticoid-induced repression while SRC1e had a tendency to reduce GR-mediated repression. These

observations uncovered the importance of highly abundant SRC1a for CRH gene regulation in the PVN.

Considering that *in vivo* the timing of activation of the two main signaling cascades involved in regulation of CRH expression can vary, in chapter 4 we tested whether this would affect CRH promoter activity. FSK-induction of the CRH promoter was efficiently suppressed by GR when dexamethasone was applied in conjunction to or rapidly after the FSK treatment. On the other hand, a short delay in GR activation resulted in a marked reduction in the ability of GR to suppress the cAMP-stimulated promoter. Since the cAMP and glucocorticoid response elements (CRE and GRE) are situated in close proximity of each other, their sterical hindrance may impair binding of the transcription factors to the promoter. Besides coregulator protein involvement, as described in chapter 3, timing of stimuli in the CRH-expressing neurons of the PVN is likely to be an important parameter for both CRH stimulation and repression.

To further study coregulator recruitment *in vivo*, in chapter 5, we evaluated the glucocorticoid-inudced leucine zipper (GILZ) gene as a potential candidate for chromatin immunoprecipitation (ChIP) assays on brain tissue. GILZ transcript was ubiquitously detected in rat brain and found to be induced after intraperitoneal corticosterone injections. So far, the GILZ promoter and the location of the GREs have been described in the context of human cell-lines. However, alignment of the proximal promoter of the rat and human gene resulted in a low homology (57%). Additionally, the previously identified GREs in the human promoter were not present in the rat sequence. Therefore, we scanned the 5kb proximal promoter of the rat gene by ChIP-assays and identified a GR-binding region. Using a position-weight matrix, we precisely localized two putative GREs in the GR-binding region. Using this method, important issues can be addressed such as coregulator recruitment by GR and MR in specific brain regions.

In conclusion, the findings presented in this thesis extend current knowledge on the neurobiology of stress and may contribute to the design of safer and more selective glucocorticoids with less side-effects. Both biological and pharmaceutical aspects have been discussed in chapter 6 along with future prospectives.