Interaction between rainbow trout corticosteroid receptors - a possible regulatory role for the mineralocorticoid receptor in response to stress?

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OR 7

The zebrafish as an in vivo model system for analysis of glucocorticoid signaling

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Upon stress, vertebrate organisms produce glucocorticoid hormones like cortisol or corticosterone. These hormones control the stress response by regulating a wide range of systems, like our metabolism growth, immune system and behavior. The effects are mediated by the glucocorticoid receptor (GR), which acts as a ligand-activated transcription factor. Here we present the zebrafish as an excellent in vivo model system to unravel the molecular mechanism of GR signaling. In this model system, just like in humans, both the GR alpha and beta-isoform occur. First, reverse-genetic studies were performed in which the expression levels of GRalpha and GRbeta were varied. Using transcriptome analysis we discovered that GRalpha regulates two distinct clusters of genes. The first cluster is regulated by GRalpha under basal conditions and contains mainly genes involved in cell cycle control and apoptosis. The second cluster is regulated upon increased activation of GRalpha and consists mainly of genes involved in glucose metabolism and proteolysis. GRbeta appears to act as a dominant-negative inhibitor of the activity of GRalpha, but is highly selective. I mainly inhibits the regulation of immune-related genes by GRalpha. Second, a forward-genetic screen was performed using the glucocorticoid-induced decrease in POMC expression in the pituitary gland as a readout. As a result of this screen four zebrafish mutants were identified that are resistant to glucocorticoid suppression of the HPA axis. Genetic identification of two of the mutants showed a mutation in the adenomatous polyposis coli (apc) gene and in the REM2 and Rab like small GTPase 1 (rsg1) gene. Both genes have not previously been associated with glucocorticoid feedback of the HPA axis, and we are currently investigating the molecular mechanism behind their involvement.

OR 8

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The role of the mineralocorticoid signaling pathways and its relationship with GR/cortisol complex in response to stress is still enigmatic in fish. In the present study, we analysed expression of corticosteroid receptors in the HIP axis of trout exposed to chronic confinement stress. We observed a sustained up-regulation of cortisol and 11-deoxycorticosterone which was associated with a negative feed-back on corticosteroid receptor gene expression (rtGR1, rtGR2, rtMR) at the level of pituitary but not in the other tissues (hypothalamus, interrenal). Moreover, we have shown co-localization of MR and GR proteins using specific antibodies in the same pituitary cells by ICC analysis. This result suggested us that, among various mechanisms, these receptors may interact through heterodimerization and regulate their activities as previously described in mammals. In order to analyse the transactivation properties of rtGR1 or rtGR2 in presence of rtMR we carried out experiments in COS-7 cells transfected with various trout corticosteroid receptors and reporter systems. Expression of rtMR in presence of rtGR1 or rtGR2 significantly decreases the cortisol-induced transactivation activity of rtGR1. When using other GRE-containing promoters, similar inhibitory effects of MR are observed. These data suggest existence of MR-GR interactions which were further studied using new mutated rtMR constructs containing different point mutations in the DNA-binding domain. Overall, our results suggest that the inhibitory effect of MR on GR activity is related to reduction of GR-self-synergy and involves N-terminal sequences of the MR. Biological relevance of such in vitro studies in relation to stress effects on HPI axis will be discussed.