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Programme and Abstracts

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THE HORMONAL RESPONSE TO STRESSORS IN FISH

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Whereas there are many similarities between the endocrine responses to stressors in the major vertebrate groups, more and more differences are reported, in particular between fishes and tetrapods. Most are related to the aquatic lifestyle of fishes. In this presentation the molecular end functional differences of the main pituitary messengers (ACTH, MSH, endorphins) and the hypothalamic peptide CRH will be compared. Subsequently control and function of cortisol will be discussed. In particular the effects of cortisol on two important target organs in fish will be dealt with: the gills and the skin. The effects on these organs are not limited to osmoregulatory processes, but also imply actions such as stimulation of the secretion of protective substances directed to pathogens, and the control of the dynamics of the main cell types of skin and gills: cortisol has important effects on mitosis, apoptosis and necrosis of chloride cells and filament cells.
A more recent finding in several species is the presence of individual differences in the release of cortisol in response to stressors (high and low responders) and the finding of behavioural differences in the stress response comparable to the active and passive coping strategies known from mammals and birds.

MOLECULAR CHARACTERIZATION OF PROLACTIN AND CORTISOL RECEPTORS IN FISH.

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In order to clarify roles of PRL and cortisol in fish osmoregulation, molecular characterization of prolactin and cortisol receptors was undertaken in Salmonids. Using a trout prolactin receptor (trPRLR) cDNA, a unique transcript (~3.4 kb) was detected by northern blot in gills, kidney and gut. Moreover, immunocytochemistry and *in situ* hybridization confirmed gill chloride cells as PRL target cells. The interaction between trout PRL and its receptor was analysed by Surface Plasmon Resonance and demonstrated the formation of a transient PRL-induced homodimerisation of the trPRLR. The instability of this PRL/trPRLR complex explains the inability to perform binding experiments using trout PRL. In addition to a first glucocorticoid receptor (GR1) cloned in trout (Ducouret et al., 1995), a second form (GR2) was recently isolated. Transactivation assay confirmed that both GR1 and GR2 were functional *ex vivo*. Moreover, a trout mineralocorticoid receptor (MR) was also identified (Colombe et al., 2000). Analysis of gene expression for these three cortisol receptors (GR1, GR2, MR) indicated their expression in osmoregulatory organs. These results clearly indicate that osmoregulation in fish depends on a complex endocrine regulation involving PRL receptor and various forms of cortisol receptors.

GLUCOCORTICOID HORMONE RECEPTOPRS IN MITOCHONDRIA OF HUMAN CELLS

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Glucocorticoid hormones regulate the transcription of nuclear genes by way of their cognate receptors. In addition, these hormones also modulate mitochondrial gene transcription by mechanisms which are as yet poorly understood. Using immunofluorescence labeling and confocal laser scanning microscopy we show that the glucocorticoid receptor of HeLa and Hep-2 cells is specifically enriched at the sites of the mitochondria which were visualized by labeling with the vital dye CMX and antibodies against cytochrome oxidase subunit I. Immunogold electron microscopy demonstrated that the receptor was located within the inner space of the mitochondria. Immunoblotting experiments also revealed the presence of glucocorticoid receptor in mitochondria isolated from HeLa and Hep-2 cells. Finally, living HeLa cells expressing green fluorescent-glucocorticoid receptor fusion protein revealed a distinct mitochondrial GFP fluorescence. Our results support the concept of a receptor-mediated direct action of steroid hormones.

INTERNALIZATION OF THE CHICKEN GROWTH HORMONE-RECEPTOR COMPLEX AND ITS EFFECT ON BIOLOGICAL FUNCTION.

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In vertebrates the main regulator of postnatal growth is growth hormone (GH), secreted in a pulsatile manner from the anterior pituitary. In plasma the majority of GH is bound to a carrier protein, termed GH-binding protein (GHBP), which appears to be identical to the extracellular domain of the GH receptor (GHR). GH binds to its receptor and mediates a cascade of cellular events. First dimerization of the GH receptor occurs, followed by the activation of a specific kinase, JAK-2. These events lead to gene transcription of insulin-like growth factor (IGF-I) and modulation of other gene expression e.g. down-regulation of the gene encoding for the type 3 (D3) deiodinating enzyme which deiodinates and inactivates T_3 in the chicken.
As a result of the episodic release pattern of GH in chicken administration of GH as a daily bolus injection does not affect growth, whereas a pulsatile GH administration does. In chicks, hypophysectomy results in an increase in hepatic GH receptors, whereas GH substitution decreases this number. Plasma concentrations of T_3 are not affected by injecting GH in intact chicks, but are decreased following hypophysectomy and restored after GH-administration. These observations led to the conclusion that as in mammals, hepatic GH-receptors can be down-regulated by GH. A direct role in gene transcription was postulated in mammals for GH, GHR and GHBP in view of their association with chromatin, subsequently to nuclear translocation through the endosomal pathway. In the present study, chicken GHR trafficking was studied and the subcellular localization of GH, GHR/GHBP was analyzed by confocal laser scanning microscopy. It was concluded that cGHR is internalized, but that GHR/GHBP are not transferred to the nucleus following internalization meaning that the biological activitiy of GH is directly generated following binding to its receptor.