# Growth hormone stimulates the alpha subunit gene expression of Na+/K+ATPase in the gills of Atlantic salmon (Salmo salar) : comparison on short and long term effects with cortisol and prolactin

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# 18th Conference of European Comparative Endocrinologists from Molecular to Integrative Biology

September 10th-14th, 1996
Palais des Congrès
Rouen - France

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## ABSTRACTS OF LECTURES AND COMMUNICATIONS

### European Society for Comparative Endocrinology Council



### Local Organizing Committee



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We have previously reported that existence of both PRL and GH within the same cells in newly hatched bullfrog *(Rana catesbeiana)* larvae, but not in tadpoles at advanced stages or adults. However, in the adult pituitary, *in situ* hybridization revealed that some of the PRL-immunopositive (ip) cells contain mRNA of GH, and some of the GH-ip cells also have PRL mRNA. On the other hand, we found that a considerable number of the enzymatically dispersed cells of the adult pituitary secrete both PRL and GH : Approximately 15 % of the GH and 8 % of the PRL blots were revealed to be derived from the same cells. Cycloheximide markedly decreased the number of blots demonstrable with both anti-GH and anti-PRL sera, but actinomysin D scarcely decreased the number of blots immunoreactive with both GH and PRL antisera. These adult pituitary results suggest that translation step for GH or PRL in the cells possessing both GH and PRL mRNAs might have been blocked and triggered by the dispersion of the cells.

## C27 ESTRADIOL 17β STIMULATES GONADAL AROMATASE ACTIVITY AND REVERSIBLE SEX CHANGE IN PROTANDROUS BLACK PORGY, *ACANTHOPAGRUS SCHLEGELI*

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The objectives was to investigate the effects of estradiol-17β ($E_2$) on gonadal function, aromatase activity, and the levels of plasma sex steroids and vitelllogenin in 2-year-old protandrous black porgy. Fish were divided into two groups, fed with control diet or diet mixed with $E_2$ (4.0 mg/kg feed) for 4 1/2 months. Significantly lower GSI was observed in the $E_2$ group. $E_2$ completely suppressed spermiation and resulted in 38 % of treated fish with developing vitellogenic oocytes in the gonad (an evidence of sex reversal). Higher gonadal aromatase activity and plasma $E_2$ levels, but lower levels of plasma testosterone (T) and 11-ketotestosterone (11-KT) were observed in the $E_2$ group. After stopping $E_2$ treatment in the early spawning season, GSI increased and spermiation were gradually resumed, increasing levels of plasma T and 11-KT, and decreasing gonadal aromatase activity and plasma vitellogenin levels were observed in the $E_2$ group. The present data show that $E_2$ induced a temporary and reversible sex change. Also, the elevated aromatase activity in gonads and $E_2$ levels in plasma, and diminished levels of plasma 11-KT associated with the occurrence of sex reversal in protandrous black porgy.

## C28 ROLE OF NITRIC OXIDE SYNTHASE AND NITRIC OXIDE IN BACKGROUND ADAPTATION IN *XENOPUS LAEVIS*

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Nitric Oxide (NO), a free radical gas, is known to be involved in many biological systems, including the anterior pituitary. NO is formed from L-arginine by the NO synthase (NOS) family of isozymes. NOS protein and mRNA have been localized in some secretory cells as well as in the folliculo-stellate (FS) cells of the rat and the human pituitary. The NOS enzyme and NO were suggested to play a role in paracrine communication with pituitary FS cells. FS cells have also been implicated to play a role in paracrine communication with the melanotropes of the pituitary pars intermedia (PI) of *Xenopus laevis* (1).

The present study was performed to evaluate the role of NOS and NO in background adaptation in *Xenopus laevis*, which is regulated by alpha-MSH secretion from the PI. Immunohistochemical staining with polyclonal antibodies against constitutive brain and inducible NOS is performed on whole brain sections of *Xenopus laevis* adapted to either a white or a black background. NOS immunoreactivity is further identified using a Western blot procedure. Moreover, during superfusion of cells from black-adapted *Xenopus* neurointermediate lobes, administration of the NO-donor sodium-nitroprusside (SNP ; 0,1 mM) together with the enzyme superoxide dismutase (SOD ; 100 U/ml), results in a 18.8 % stimulation of basal alpha-MSH secretion. Perifusion of the melanotropes with the NO scavenger hemoglobin (1.3 μM) has no effect on basal alpha-MSH secretion but prevents SNP-stimulated secretion. The role of NOS activity in plasticity of the PI during background adaptation in *Xenopus laevis* is presented for discussion.

(1) H.P. de Koning, B.G. Jenks, W.J.J.M. Scheenen, E.P.C.T. de Rijk, R. Caris and E.W. Roubos. Neuroendocrinology 54 : 68-76 (1991).

## C29 GROWTH HORMONE STIMULATES THE α SUBUNIT GENE EXPRESSION OF $Na^+/K^+$ATPase IN THE GILLS OF ATLANTIC SALMON *(SALMO SALAR)* : COMPARISON ON SHORT AND LONG TERM EFFECTS WITH CORTISOL AND PROLACTIN

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$Na^+/K^+$ATPase activity in Atlantic salmon gills is under control of endocrine factors closely linked to the fish environmental salinity and developmental stage. However, it is not known whether these effects are associated to changes in gene expression or activation of existing protein. To clarify some of the pathways of $Na^+/K^+$ATPase regulation in the gill tissue, we have studied in Atlantic salmon the gene expression of the α subunit of the enzyme looking at short (0 to 24 hours) and long term effects (0 to 15 days) of growth hormone, cortisol and prolactin. For this purpose, two cDNAs encoding a conserved or a variable region of the trout α subunit were isolated after PCR amplification. For the short-term regulation the fish were given one injection of oGH (2 μg/g) or oPRL (2 μg/g) or cortisol (5 μg/g) or just saline solution for controls. For the long-term effect a cholesterol implant of oGH (250 μg/fish), or oPRL (250 μg/fish) or sham and a silastic implant of either cortisol (800 μg/fish) or control were used. Results showed no alteration in gene expression during the time comprised in the 24 h period. Neither the mRNA levels nor the enzyme activity were significantly different. In the long-term study, GH stimulated both a subunit mRNA levels and enzyme activity after 12 days in FW. This was also shown after the SW tansfer of these fish but activity levels were several fold higher. PRL slightly increased $Na^+/K^+$ ATPase activity but not transcript levels in FW fish. We saw a salinity-dependent stimulation in both PRL and control fish. Cortisol implants failed to elicit any type of response. Neither treatment lead to the appearance of a new transcript as only a single 3.7 kb mRNA was detected by Northern blot analysis.

## C30 THE GENES CODING FOR THE GTH β SUBUNITS OF TWO TELEOSTS, BLACK CARP AND TILAPIA

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Two model fish, black carp *(Mylopharyngodon piceus)* and tilapia *(Oreochromis mossambicus)*, were chosen for the study of gonadotropin gene regulation. The first representing a late maturing cyprinid with a synchronous ovary and the second, an example of an early maturing perciform with a batch synchronous ovary. As a first step towards the identification of the gonadotropin regulatory sequences, we have set out to isolate the genes coding for the gonadotropin β subunits. The black carp (bc) β-GtHII gene was isolated from a bc genomic DNA library using the homologous β-GtHII cDNA as a probe. The deduced amino acid sequence coding for the mature peptide shows 100 % homology to that of the silver carp, 99 % to that of the grass carp and 96.5 % to that of the common carp. A small intron of 99 bp was identified three amino acids downstream from the fifth cystein residue, a position strictly conserved in all the β subunit genes identified so far. Southern blot analysis of bc genomic DNA using the β-GtHII cDNA sequence as a probe indicates that only a single copy of the gene exists. The genes coding for the β subunits of GtH I&II from tilapia were isolated from a tilapia genomic library (Swennen *et. al.*, DNA and Cell Biol. 1992). The gene coding for β- GtHI was identified using the gilthead seabream β-GtHI cDNA sequence as a probe. The deduced amino acid sequence obtained showed it to be 58 % homologous to that of the gilthead seabream and 70 % homologous to that the bonito. The gene coding for the β-GtHII subunit was isolated using a tilapia β-GtHII cDNA probe. Both genes appear to contain a large intron at the conserved position of three amino acids