Comparaison of the biological potencies of the two tilapia prolactins on tiPRL receptor transfected in human fibroblastes (293) cell line

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ABSTRACTS OF LECTURES AND COMMUNICATIONS

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the oogenesis from the oogonia to the mature eggs, based on differences of staining, size and on nucleus and cytoplasm structure, as viewed through the ligth microscope. Three of these stages correspond to the first or previtellogenic phase and the other three to the second or vitellogenic phase. Several types of atresic eggs are distinguished which probably represent different phases in the process of atresia. The testicular tissue belongs to the continuous or unrestricted spermatogonial type (as classified by Grier, 1981), and forms a complex network of seminiferous tubules.

CONTROL OF GENE EXPRESSION

P148 BONE GLA PROTEIN IN FISH : MOLECULAR CLONING AND DEVELOPMENTAL APPEARANCE IN EARLY LARVAL STAGES OF THE SEA BREAM *SPARUS AURATA*

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Bone Gla Protein (BGP) is a bone-specific protein isolated at the protein level from a variety of species from fish to mammals but its gene structure and control of expression have been determined exclusively in mammals. Our objectives were to clone the BGP cDNA and study its developmental appearance in a non-mammalian vertebrate. Sea bream larvae 12 to 90 days old were collected and development of cartilage and bone structures was followed by histological techniques. Calcification was found to be complete by 60 days. Total RNA from fully calcified larvae was reverse transcribed and then amplified by PCR using a 3' oligo dT primer and a degenerated 5' primer designed according to aminoacid 23 to 30 of the sequence of the mature seabream BGP protein previously purified (1). The 325 bp cDNA thus obtained was sequenced revealing an open reading frame corresponding to the aminoacid sequence previously obtained for the seabream BGP in this region of the protein and extending 252 bp of 3' non-coding region from the stop codon to the insertion of the poly(A) tail. The presence of BGP mRNA was followed throughout the early stages of development of seabream by in situ hybridization using an antisense RNA probe. Detection of specific hybridization paralleled appearance of the first calcified structures : the mandibulary region, the skin (due to the presence of calcified scales) and sites of fin insertion. Unexpectedly, specific BGP labeling was also seen in non calcified structures, particularly in brain and gill bars, suggesting that BGP is present in other than calcified structures in fish. Alternatively, it could indicate the presence of other protein(s) of the same gene family with distinct patterns of expression. This possibility is currently being investigated. (1) M.L. Cancela, M.K. Williamson, P.A. Price. Int. J Peptide Protein Research, 46 :419, 1995.

P149 ANALYSIS OF A SALMON PROLACTIN PROMOTER IN TRANSGENIC RAINBOW TROUT

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A gene encoding for the chinook salmon prolactin has been recently isolated and fully sequenced by Xiong et al. (1992). The purpose of this study was to demonstrate the cell specific expression driven by cis-regulatory elements of the salmon PRL gene in transgenic rainbow trout. For that purpose, stable lines of transgenic fish having integrated the foreign gene were produced. Fish harbored a fusion gene containing 2.4 Kb of the 5' flanking sequence (Xiong et al., 1992) and the reporter gene, lac Z encoding β-galactosidase protein.

This construct was retained in approximatively 30 % of 9 months-old fish derived from micro-injected eggs and induced a very low expression : analysis of β-galactosidase activity showed that only a few PRL cells expressed the reporter gene in two fish among 28 positives. However these first results agreed with a cell specific expression. In order to exclude the problem linked to mosaic integration of the transgene, we produced 6 F_1 families from wild-type females mated with F_0 transgenic males. The percentage of inheritance varies between families (17.5-28 %). Interestingly, β-gal activity was detected in pituitary gland from different animals with a variable intensity, but only in PRL cell. Southern blot analysis indicate that multiple copies, often organized in concatemers, were integrated into the genomes. Fifteen different patterns have now been identified. For each of them we produce the F_2 generation. In some of them, we confirmed stable inheritance of the transgene and specific expression of β-Galactosidase in PRL cells. A comparative analysis of β-gal expression for each of these integration pattern in our different F_2 families is currently been performed.

P150 *IN VITRO* STUDY OF THE TSH SUBUNIT mRNA REGULATION IN PRIMARY CULTURE OF PITUITARY CELLS OF THE EUROPEAN EEL, *ANGUILLA ANGUILLA*

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Recent *in vivo* studies demonstrated that both T_3 and T_4 down regulate TSH subunit mRNAs in the European eel at the silver stage. To study the direct action of the thyroid hormones (TH) at the pituitary level we inititiated *in vitro* experiments on pituitary cells in primary culture.

Dot-blot analysis showed a 2.5 fold increase in TSH β mRNA in control cells between the first and 14th day of culture, which probably reflects the abolition of in vivo inhibition either by circulating TH or by negative regulation exerted by the hypothalamus. Addition of T_3 or T_4 to the culture medium decreased TSH β mRNA of about 70 % compared to day 14 control cells, indicating that both TH are able to down regulate TSH β mRNA expression through direct action at the pituitary level.

To increase the sensitivity of the detection and quantification of the TSH mRNA we developed RNAse protection assays (RPA) for the α and TSH β subunit mRNA. These RPAs are used to further characterize the negative feed-back of TH on TSH mRNA expression as well as investigate the role of brain neuromediators in the regulation of TSH mRNA.

P151 COMPARISON OF THE BIOLOGICAL POTENCIES OF THE TWO TILAPIA PROLACTINS ON tiPRL RECEPTOR TRANSFECTED IN HUMAN FIBROBLASTES (293) CELL LINE

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Tilapia has served as an important model system for understanding PRL functions in teleosts. Two forms of prolactin ($tiPRL_I$ and $tiPRL_{II}$) with only 69 % sequence identity have been shown to be produced by the pituitary prolactin cell, $tiPRL_I$ being more similar to other fish prolactins. Although the two PRLs have been shown to have different potencies in various fonctions in tilapia, only one form of receptor have been cloned and detected in the various tissues in this species. The biological significance of the two PRLs being unknown, further analysis at the molecular level on signal transduction pathways was undertaken. Because the PRL receptor in fish as well as in mammals belongs to the cytokine receptor family, it was of interest to determine if the transduction pathway already described using PRLR in mammals also apply to fish RPRL. tiRPRL was cotransfected in 293 cells with the STATS responsive region (tK-LHRR-luc). All biological activities were normalized against constitutive cotransfected β-gal activities. A chimeric short form of tiPRL receptor was constructed and used as a negative control. Recombinant tiPRLs ($tiPRL_I$ and $tiPRL_{II}$) were a gift of Dr F. Rentier (Leuwen). Comparaison of the potencies of the two forms of tiPRL and oPRL showed good agreement between results obtained by radioreceptor assays and biological activities : oPRL = tiPRL-I > tiPRL-II.

Our data support the hypothesis that at least one of the signaling pathways of PRL action in fish might implicate JAK2 and STAT5.

P152 ONTOGENY OF GROWTH HORMONE AND PROLACTIN GENES EXPRESSION IN EARLY STAGES OF THE GILTHEAD SEABREAM, *SPARUS AURATA*

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In some mammal, avian and piscine species, studies have shown that Growth Hormone (GH) and Prolactin (PRL) are important in early ontogeny. In the present study we describe the expression of GH and PRL in sea bream larvae at different developmental stages. Total RNA was extracted from larvae sampled daily (up to 11 days). Reverse transcriptase was used to synthesize cDNA. Expression of GH and PRL genes was determined by PCR, with primers based upon the sequence of these genes in sea bream adults. The identity of the PCR products were validated by sequencing and PCRs were followed by Southern blot analysis using ^{32}PdCTP labeled GH and PRL probes. Specific hybridization for GH was seen after day 2 posthatching with a dramatic increase after day 6. PRL showed much lower levels of expression and was first seen in day 2, increasing slightly to day 11. The expression of βActin, used to normalize the relative amounts, was high from the first day and levels were constant throughout the next 10 days. These results seem to suggest that the pituitary gland, despite its stage of differentiation, is already able to produce the mRNA for two kind of hormones, known to have, in the adult, different cell origins. The levels of GH also show a fairly high transcription rate. It is interesting that the increase in GH mRNA coincides with the change from yolk dependence to a free feeding larvae (4 to 6th day).

P153 EFFECTS OF VASOPRESSIN ON EXPRESSION OF THE mRNA FOR POMC IN OVINE ANTERIOR PITUITARY CELLS

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Although arginine vasopressin (AVP) increases synthesis and secretion of ACTH by anterior pituitary (AP) cells, evidence to date suggests that AVP has either no effect or decreases mRNA for POMC, the biosynthetic precursor of ACTH. Because corticotrophs are heterogeneous and subject to intrapituitary regulation, we measured the effects of elimination of CRH-target cells and the presence or absence of AVP on the expression of POMC mRNA in cultured ovine AP cells (N = 7, every treatment in all experiments). CRH-target cells were eliminated by treatment with a CRH-toxin conjugate (Cx). Collagenase-dissociated AP cells were washed, cultured 24 h, exposed to either vehicle or Cx for the next 18 h, washed, cultured for 3d more, washed and after equilibration to serum-free conditions, incubated 4-5 h ± AVP (100 nM, treatments in duplicate). POMC mRNA was measured in cells by northern blot quantification ; ACTH in medium and cell extracts was determined by RIA. POMC mRNA expression was increased by AVP in Cx-treated cells (by 20 ± 6 %) and, surprisingly, in intact populations (by 48 ± 13 %) as well. Elimination of CRH-targets itself was also associated with an increase in POMC mRNA (by 70 ± 12 %). These data suggest expression of POMC mRNA in corticotrophs can be stimulated by AVP and is also subject to intrapituitary influences.

P154 ECDYSTEROID DEPENDENT TISSUE DIFFERENTIATION AND SYNTHESIS OF CYTOSKELETAL ELEMENTS IN AN EPITHELIAL CELL LINE FROM *CHIRONOMUS TENTANS*

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Ecdysteroids induce differentiation processes in the permanent epithelial cell line from *Chironomus tentans*, which grows as homogenous monolayered multicellular vesicles. At the beginning morphogenetic changes are observed in patches of cells, but lateron the whole vesicle is engaged in the differentiation process. As a first sign an enhanced concentration of ecdysteroid receptors and muscarinic acetylcholine receptors is observed in these patches. In addition there is a transient increase in the concentrations of actin and tubulin and the mRNA's for these proteins. The relation of filamental to globular actin remains constant.

CELL SIGNALLING

P155 SIGMA LIGANDS STIMULATE THE ELECTRICAL ACTIVITY OF CULTURED FROG PITUITARY MELANOTROPHS

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In amphibians, the pars intermedia is innervated by nerve fibers containing dopamine, GABA and various regulatory peptides. Most of these classical neurotransmitters and neuropeptides are involved in the regulation of the electrical activity and the secretion of α-MSH by pituitary melanotrophs. In the present study, we investigated the effect of sigma ligands on the electrophysiological activity of frog melanotrophs in primary culture. A brief application of 1,3-ditolylguanidine (DTG) or (+)-pentazocine (10 μM) induced a transient depolarisation associated with a concomitant increase of the action potential frequency. In voltage clamped cells, these two sigma figands evoked an inward current which increased with depolarizing potentials. The reversal potential of the current corresponded to the K^+ equilibrium potential. The voltage-activated delayed rectifier K^+ current evoked by depolarizing pulses from – 50 to + 50 mV was reduced by 23 % in the presence of DTG or (+)-pentazocine (20 μM). In cells dialysed with GTP-γ-S (100 μM), a brief exposure to DTG caused an irreversible inward current. Replacement of GTP by the non-phosphorylysable analogue GDP-β-S (100 μM) inhibited the DTG-induced current Incubation of the cells with cholera toxin (18-24 h, 1 μg/ml) 1 μg/ml abolished the sigma-evoked currents, whereas pre-treatment with pertussis toxin had no effect. The present data show that sigma ligands stimulate the electrical activity of frog melanotrophs by inhibiting a voltage-activated K^+ conductance through a cholera toxin sensitive G-protein. *Supported by grants from the IRJ and EU (HCM # ERBCHRXCT 920017).*

P156 CYCLIC-AMP EGRESS BY THE *XENOPUS* NEUROINTERMEDIATE LOBE IS ELICITED BY LOW Ca^{2+} AND THE ADENYLYL CYCLASE INHIBITOR SQ 22,536

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Previously we have shown that a stimulated cAMP egress from *Xenopus* neurointermediate lobes occurs during inhibition of aMSH secretion by baclofen ($GABA_B$ agonist) and dopamine (1). We have now further characterized this egress. It can also be elicted by lowering the Ca^{2+} concentration in the medium. At 10^{-8} M Ca^{2+} a strong egress of cAMP was found, which was completely reversible. This cAMP egress was inhibited by the transport inhibitor bromosulfophtalein (BSP) but not by the inhibitor probenecid. The adenylyl cyclase inhibitor SQ 22,536 caused a strong cAMP egress (factor 8). This egress was not BSP-sensitive.