Molecular characterization of prolactin receptor (PRLR) in fish

Olivier Sandra, Philippe Le Rouzic, Benoît Aupérin, M. Edery, P.A. Kelly, Patrick Prunet

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

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

ABSTRACTS OF LECTURES AND COMMUNICATIONS

European Society for Comparative Endocrinology Council

President :	A. De Loof, Belgium
Vice-President :	H. Vaudry, France
Secretary-Treasurer :	E.W. Roubos, The Netherlands
Honorary Vice-President :	F. Gracia-Navarro, Spain
Members :	X. Bellès, Spain ; D. Larhammar, Sweden ; G.M. Coast, United Kingdom ; R. Pierantoni, Italy ; S. Dufour, France ; A.M. Polzonetti-Magni, Italy ; W.P.M. Geraerts, The Netherlands ; F. Sehnal, Czech Republic ; J. Koolman, Germany

Local Organizing Committee

Chairman :	H. Vaudry
Members :	V. Carpentier, L. Cazin, N. Chartrel, V. Contesse, J.M. Danger, C. Delarue, L. Desrues, M. Feuilloley, S. Jégou, M.K. Kodjo, M. Lamacz, F. Leboulenger, I. Lihrmann, E. Louiset, A.G. Mensah-Nyagan, M. Montéro, I. Remy-Jouet, M.C. Tonon, H. Tostivint, L. Yon

---

therms is still scarce. The first studies on insulin binding, performed in muscle of salmonids resulted in very low number of receptors (82 ± 23 fm/mg ; Kd : 0.38 ± 0.02 nM) one tenth of those found in mammals. Studies in omnivorous fish species reveal higher number of receptors (440 ± 47 fm/mg, in carp).
When IGF-I binding in fish muscles was studied, higher number of IGF-I receptors (770 + 190 fm/mg, in carp), with higher affinity (Kd : 0.26 ± 0.06 nM) and specificity than insulin receptors were found in all species studied. This finding was checked in amphibian and reptiles and similar insulin/IGF-I binding ratio (0.51-0.73) was found in all ectotherms. The situation was the opposite in birds and in mammals, in which predominance of insulin receptors in muscle was found (1.68-3.12, insulin/IGF-I binding ratio). So, future questions to address are : do insulin and IGF-I have different functions in muscle during vertebrate evolution ? and what is the significance of the high number of IGF-I receptors in muscle tissues of fish ?
Supported by PB94-0864 and GRQ94-1051 (CIRIT).

S15 PECULIARITIES OF INSULIN SECRETION IN CHICKENS

N. Rideau

Endocrinologie de la Croissance et du Métabolisme, Station de Recherches Avicoles, INRA, 37380 Nouzilly, France.

The origin of the hyperglycaemic state (as compared to mammals), the mechanism of insulin release and the possible implications of glucose-insulin balance in the control of growth and body composition are poorly understood in bird species. In contrast to mammals, very high glucose concentrations are required to induce a typical biphasic insulin release from the chicken isolated and perfused pancreas. We have further investigated the stimulus secretion coupling and shown that most of the nutrients which are « primary initiators » of insulin release in mammals : D-glyceraldehyde (D-GA), D-mannose, L-leucine and α-ketoisocaproate (KIC) are either poorly or not efficient at initiating insulin release when perfused alone. An additional but noninsulinotropic fuel supply permits to those nutrients to become insulinotropic in the chicken pancreas. Glucose is more efficient than other nutrients (D-GA, L-glutamine and L-asparagine) to exert this « permissive » effect. An intracellular metabolism is required : 3-O-methyl-D-glucose does not potentiate the response to KIC. The relative insensitivity of the chicken pancreas can also be overcome by the simultaneous perfusion of cAMP and/or acetylcholine, according to the nutrient. On the other hand 10-40 mM K^+ or 20 mM arginine induce a rapid monophasic insulin output. Together, these results suggest that the metabolic threshold which permits the switching of the β-cell from the resting to the active state is much higher in chicken than in mammals. By an unknown mechanism, the threshold is lowered more efficiently by glucose than by the other fuel nutrients. Potentiation mechanisms and membrane depolarisation events are present in the chicken pancreas. Studies aimed at understanding the peculiarities of the chicken β-cell are presently performed using isolated chicken islets of Langerhans.

S16 REGULATION OF CHICKEN MUSCLE GROWTH BY INSULIN-LIKE GROWTH FACTORS

M.J. Duclos

INRA, Recherches Avicoles, 37380 Nouzilly, France.

The Insulin-like Growth Factors (IGF) stimulate all stages of muscle growth : multiplication and differentiation of myogenic precursors, anabolism of myotubes and muscle fibers. During posthatch growth of the chick, incorporation of nuclei into muscle fibers is an important process which implies the recruitment of a specific set of myogenic cells, termed muscle satellite cells. These cells can be isolated enzymatically from chick pectoralis muscle and cultured *in vitro*. Both IGF-1 and -2 stimulate DNA synthesis by these cells, this effect is mediated through a unique IGF receptor type with characteristics corresponding to those of the type 1 receptor described in mammals. Satellite cells from chickens selected for high (HG) or low growth rate (LG) have been compared. Satellite cells isolated from HG chicks are more responsive to IGF-1 than cells from LG chicks. IGF binding proteins which are secreted at very low levels in satellite cell cultures do not explain this difference which must result from differences at receptor or postreceptor levels. Following solubilization of muscle membranes or muscle homogenates and lectin affinity chromatography, IGF binding can be detected. Muscle IGF receptor number decreases with age, but does not differ between HG and LG chickens. The IGF receptor kinase activity toward the artificial substrate poly Glu-Tyr (4 :1) is similar in both lines. Our data show that differential responsiveness to IGF-1 might partly account for genetic differences in growth rate in this species and suggest a critical role for IGF in the regulation of muscle growth in the posthatch chick. This difference, however cannot be simply explained at the receptor level.

MEMBRANE RECEPTORS AND THEIR MECHANISMS OF ACTION

S17 PROLACTIN SIGNALING THROUGH STAT PROTEINS IN MAMMALS AND NON-MAMMALIAN VERTEBRATES

B. Aupérin, N.S. Nevid and N.D. Horseman

Department of Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, Ohio 45267-0576, USA.

Prolactin (PRL)-induced activation of STAT (**S**ignal **T**ransductor and **A**ctivator of **T**ranscription) proteins was compared among different vertebrate groups. STAT activation was studied by western blot, to assess phosphorylation, and by Electrophoretic Mobility Shift Assay (EMSA), to assess their binding activity to the inverted repeat GAS (IRG) element found in the promoter of the transcription factor IRF-1 (Interferon Regulatory Factor-l).
This study showed that immunoreactive Stat 1 and Stat 5 are present in representative species from four vertebrate classes including fish (tilapia gills), amphibians (newt skin), birds (pigeon crop sac and liver) and mammals (rat Nb2 cells and human 2ftGH cells). Prolactin treatment induced a clear phosphorylation of Stat 5 in all the species and tissues tested and in 2ftGH cells transfected with pigeon PRL receptor (PRL-R) and Stat 5 cDNAs. Stat 1 phosphorylation was induced by PRL in pigeon crop sac and liver, and in rat Nb2 cells. EMSA analysis revealed two different types of PRL-induced complexes. A slowly migrating Stat 5-containing complex was observed with protein extracts from tilapia gills, pigeon crop sac, Nb2 lymphocytes and in transfected 2ftGH fibrosarcoma cells. A faster migrating Statl-containing complex was also observed with extracts from pigeon liver and Nb2 cells.
To compare the activities of PRL-R from different species within the same cellular system, COS-7 cells were transfected with Stat 5 and Stat 1 cDNAs and with tilapia, pigeon or bovine PRL-R cDNAs. oPRL treatment of these transfected cells induced Stat 1 and Stat 5 complexes, but with different oPRL sensitivities. This study shows that the transduction of the PRL signal involves Stat 1 and/or 5 in all the species tested. However, the differences observed in the binding activity between pigeon crop sac and liver and the formation of both complexes in PRL treated transfected COS cells suggests that coactivators and/or repressors can influence STAT protein binding activity.

S18 MOLECULAR CHARACTERIZATION OF PROLACTIN RECEPTOR (PRLR) IN FISH

O. Sandra (1), P. Lerouzic (1), B. Aupérin (1), M. Edery (2), P.A. Kelly (2) and P. Prunet (1)

(1) Laboratoire de physiologie des Poissons, INRA, Rennes, France, (2) Laboratoire d'Endocrinologie Moléculaire, U344, INSERM, Paris, France.

PRL is implicated in many physiological actions in vertebrates. However in fish, osmoregulation is the most studied function for PRL and scattered litterature exists about direct implication of PRL in other physiological process. In order to improve knowledge about PRL effects, we have studied PRL receptor in tilapia *(Oreochromis niloticus)*. Using the two forms of tilapia PRLs ($tiPRL_I$ and $tiPRL_{II}$) as ligands in homologous radioreceptor assay, one single high-affinity site has been characterized in gill, kidney and intestine, confirming the major role of PRL in the control of osmoregulation. Liver and skin display a low but specific binding to both tiPRL forms, suggesting possible involvement of PRL in metabolism and ectoderm

proliferation. By using an expression cloning approach, a cDNA encoding a tiPRLR similar to the long form of mammalian PRLR was identified and allowed us to further investigate at another molecular level. Northern-blot performed in osmoregulatory tissues from fresh water or brackish water show only one transcript (~ 3.2 Kb), arguing rather for the existence of only one binding site. *In situ* hybridization is presently performed in these tissues to define the cellular localization of the transcript. Furthermore, a single transcript of the same size is found in other tested tissues as skin, liver, gonads, brain, and haemopoietic organs (spleen and anterior kidney). These results suggest implication of PRL in reproduction, behavior and immunity. Finally the regulation of tiPRLR transcript in various organs was also studied in two physiological situations : during a transfert from fresh water to brackish water and during confinement stress. Altogether these results confirm the versatility of PRL actions in this fish species where they appeared to be as diversed as in higher vertebrates.

S19 INTERACTION OF LACTOGENIC HORMONES WITH PROLACTIN RECEPTORS

A. Gertler, J. Grosclaude and J. Djiane

Faculty of Agriculture, The Hebrew University, Rehovot 76100, Israel, and Institut National de la Recherche Agronomique, 78352 Jouy-en-Josas Cedex, France.

Recombinant prolactin receptors extracellular domains (PRLR-ECDs) from rabbit (rb) and bovine (b) mammary glands and rat (r) liver were expressed in *E. coli,* refolded and purified to homogeneity. Interaction of PRLR-ECDs with hGH, several PRLs and bovine placental lactogen was studied utilizing surface plasmon resonance (SPR) methodology which enables real-time kinetic measurements of the interactions, calculation of kinetic constants and stoichiometry of interaction even in cases of very transient interactions occur. In contrast to gel-filtration or crystallographic studies whereas in most cases the interaction of PRLR-ECDs with various lactogenic hormones indicated formation of 1 :1 complexes, SPR experiments indicated that in *all cases* a 2 :1 complex was formed. In majority of interactions (and in particular in homologous interactions) the 2 :1 complex was very unstable and underwent rapid dissociation to 1 :1 complex. We suggest that like in GHRs, the activation of PRLRs occurs also through hormone-induced receptor dimerization and that transient dimerization of the receptor lasting few seconds, may be sufficient to initiate the biological signal. Once the signal is initiated, further existence of receptor dimer is no longer required. Its fast dissociation to 1 :1 complex or to its components may be even advantageous as it permits activation of additional receptors.

S20 ISOLATION AND CHARACTERIZATION OF THE CHICKEN INSULIN RECEPTOR SUBSTRATE-1 GENE

M. Taouis

Endocrinologie de la Croissance et du Métabolisme, INRA, SRA, 37380 Nouzilly, France.

The action of insulin is mediated via the insulin receptor, a tyrosine kinase. Upon ligand binding, this receptor undergoes autophosphorylation and its kinase becomes activated, phosphorylating cellular substrates. The major substrate is the insulin receptor substrate-1 *(IRS-1). IRS-1* acts as a docking protein and mediates multiple interactions with other proteins, resulting in transduction of the metabolic and mitogenic signals of insulin. Up to now, the *(IRS-1)* gene has been cloned from in species (human, rat, mouse and frog). In the present study, the chicken *(IRS-1)* gene was cloned. Chickens have a higher blood glucose than mammals in both the fasting and the fed state. Chicken *(IRS-1)* DNA sequence encodes a 1240 amino acid protein. The most conserved regions were the IRS homology-2 (IH-2), the pleckstrin homology, and the shc and *(IRS-1)* NPXY-binding (SAIN) domains. Twelve of the cIRS-1 tyrosine residues are in sequence motifs that, when phosphorylated, could interact with proteins containing SH2 domains. All twelve of these motifs are conserved. *(IRS-1)* mRNA is expressed during embryogenesis in chicken and persists after hatching. In LMH cells, derived from a chicken hepatoma, two bands were tyrosine phosphorylated in an insulin-dependent manner : *(IRS-1)* (~ 180 kDa) and the insulin receptor β-subunit (~ 95 kDa). Therefore, chicken *(IRS-1)* is structurally and functionally similar to its human homolog, despite the evolutionary distance between birds and mammals.

ENDOCRINE AND PARACRINE REGULATION OF MINERALIZATION PROCESS

S21 ON THE ORIGIN OF METAZOAN CALCIFICATION

P. Westbroek and F. Marin

Netherlands School of Sedimentary Geology, Gorlaeus Laboratoria, Postbus 9502. 2300 RA Leiden, The Netherlands.

Mucous materials excreted by external epithelia of the metazoans form highly diverse macromolecular mixtures providing binding sites for potentially harmful invaders such as viruses and bacteria. We have evidence showing that in the mucus of marine invertebrates special moieties are capable of specifically trapping and binding calcium carbonate crystals and their nuclei. Removal of these crystalline materials, e.g. by ciliary activity, would protect the soft tissues from spontaneous mineral overgrowth when the supersaturation of the ambient water is sufficiently high. We propose the term anti-calcification to denote this protective function.
We suggest that the sudden appearance of calcified skeletons among many different invertebrate taxa at the Precambrian - Cambrian transition may have required minor reorganizations of pre-existing secretory functions. In particular, factors of the skeletal organic matrix responsible for regulating crystal growth by inhibition may be derived from the anti-calcifying mucous excretions. We tested this hypothesis by comparing the serological properties of skeletal water-soluble matrices (SM) and mucous excretions of three invertebrates — the scleractinian coral *Galaxea fascicularis,* and the bivalve mollusks *Mytilus edulis* and *Mercenaria mercenaria.* Cross-reactivities recorded between muci and SM suggested that these different secretory products have a high degree of homology. Furthermore, freshly extracted muci of *Mytilus* were found to inhibit calcium carbonate precipitation in solution.

S22 PROTEINS CONTROLLING BIOMINERALIZATION TO FORM THE HIGH-PERFORMANCE MICROLAMINATE COMPOSITES OF ABALONE SHELL

A.M. Belcher, C. Zaremba, P.K. Hansma, G.D. Stucky and D.E. Morse

Departments of Molecular, Cellular & Developmental Biology ; Chemistry ; Physics ; and Materials, University of California, Santa Barbara, CA 93106, USA.

Nature uses two different mechanisms for directing structure during biofabrication of the high-performance microlaminate composites of calcium carbonate and protein forming the abalone shell and flat pearl. One controls structure and orientation of the mineral at the atomic and nanoscale levels ; the other controls ordering over macroscopic dimensions. Proteins of 3 families exert this control : (1) a crystal-nucleating protein ; (2) polyanionic proteins that determine the phase, orientation and morphology of calcite and aragonite ; and (3) matrix envelope proteins that determine the lamellar spacing and crystal size of aragonite in nacre. The nucleating protein sheet, in conjunction with calcite-specific polyanionic proteins, direct nucleation of highly oriented calcite to form a « primer » in the first step of biomineralization. The abrupt subsequent transition to aragonite is controlled by cell recognition of the primer surface, activating a genetic switch to production of polyanionic proteins that direct the synthesis of aragonite. Microlaminate nacre is organized over macroscopic dimensions by continuous crystallization of the atomically coherent protein-oriented aragonite through a network of pores in the multilayered protein matrix sheets. Preliminary sequence analyses of the proteins and their cloned genes reveal several unusual structural features. Use of the purified nucleating and polyanionic proteins allows us, *in vitro,* to abruptly and sequentially switch crystallographic phase from calcite to aragonite and vice-versa, producing multiphase composites with micron-scale domains.