Prolactin signaling through stat proteins in mammals and non-mammalian vertebrates

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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 ?
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S15 PECULIARITIES OF INSULIN SECRETION IN CHICKENS

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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

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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

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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-1).
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

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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