CALCIUM, THE BACKBONE OF FISH CULTURE: IMPORTANCE IN SKELETAL FORMATION, REPRODUCTION AND NORMAL PHYSIOLOGY (Q5RS-2001-02904)

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A general objective of the FISHCAL project was to identify the relative importance of dietary, endogenous and environmental sources of calcium during critical phases of development, growth and reproduction of sea bream during a normal production cycle in the fish farm. A specific objective was to determine experimentally the roles of parathyroid hormone related protein (PTHrP) in whole animal calcium homeostasis. In addition the molecular mechanisms that underpin bone formation in fish (sea bream and sturgeon) and the way in which PTHrP regulates this process was studied.
The project has developed a series of tools to study calciotrophic hormones which included: a) recombinant PTHrP for bioassay studies; b) antisera against different regions of the PTHrP protein molecule for immunocytochemistry, c) radioimmunoassays against different regions of the PTHrP protein molecule; d) quantitative polymerase chain reaction (Q-PCR) assays for PTHrP, its receptors and calcium-sensing receptor and e) probes of the same molecules for in situ hybridization; f) a 2000 clones cDNA microarray to identify PTHrP responsive genes.
It was established that the rate of calcium uptake by sea bream larvae is directly related to fish size and calcium water content. Salinity strongly influences the route of uptake partly through an effect on drinking. PTHrP increased whole body calcium uptake in larvae of at least up to 80 mg. A decreased calcium and phosphorus accumulation was found when calcium is limited in water and/or diet, resulting in growth arrest when calcium is limited in both water and diet. Similar observations were made in sturgeon. Vitamin D has an important role mediating calcium metabolism in sea bream. Immunocytochemistry and in situ hybridisation analysis showed that in Sparus juveniles, PTHrP and its receptor is abundantly expressed in the epidermis and skin. Expression was low in kidney tubules, interrenal tissue and the epithelia of the gut but the leucocytes in the lamina propria hybridised abundantly with all the PTHrP probes and that of the receptor. Interestingly in larvae with skeletal abnormalities a change in PTHrP expression was found in cartilage and bony structures. The preceding observations taken together with the pivotal role PTHrP has in cartilage formation in mammals, suggest this will be an important area to study in skeletal abnormalities in fish.
A single class of binding sites was found in isolated sea bream enterocyte membrane preparations. Amino-terminal deletions in teleost PTHrP, such as removal of up to the first six residues (7-34)PTHrP do not affect the binding ability to enterocyte membrane fractions. RT-PCR using specific primers amplified PTH 3 receptor (PTH3R) and not PTH1R. Receptor signalling occurs via the adenylyl-cyclase AC/phosphokinase C (AC/PKC) pathway and not the phospholipase C /inositol phosphate (PLC/IP) pathway. A single class of binding sites was identified in fish scales. Aminoacid deletion studies yielded similar results to enterocytes, but, unlike the enterocytes, both intracellular signalling pathways are active and only PTH1R was detected by RT-PCR. Nevertheless PTHrP induced TRAPC activity could be inhibited only by SQ-22536 a blocker of the AC/PKA signalling pathway indicating a role of this pathway in ossification.
PTHrP induced cortisol production in interrenal cells in vitro dose dependent fashion. Truncated forms of Fugu (1-34)PTHrP in which the first (2-34), first two (3-34) or first

six amino acids (7 34) had been removed failed to stimulate cortisol release from head kidney. These results indicate that amino-terminal deletions of teleost PTHrP affects the ability of these truncated forms to stimulate in vitro cortisol release from interrenal tissue. The effect of PTHrP is specific and through one of the two PTH receptors, PTH1R and PTH3R, present in interrenal cells.

Among several PTHrP responsive genes, osteonectin (OSN), was isolated from an intervertebral sea bream cDNA library. Semi quantitative RT-PCR indicated that sbOSN is most abundant in calcified tissues and the scales, intervertebral disc and the vertebrae have the highest level of expression, followed by the caudal rays, branchial arches and opercular bone. In scales, OSN mRNA is abundantly expressed in the scleroblasts present at the interface between the fibrous and the bony layer but appeared to be absent from the overlying epidermis. In the skull, bones localized in the anterior-frontal region of the neurocranium showed affinity for alcian blue 8GX as well as for van Gieson solution confirming it is composed of both cartilaginous and osseous tissues. OSN mRNA is abundant in the presumptive chondrocytes at the transition layer between cartilage and bone and also in some scattered presumptive chondrocytes of this tissue.

A series of studies in Acipenser naccarii determined a hypercalcemic action of PTHrP although levels of the hormone were apparently lower than those found in teleost fish.