Report for 2002KY2B: Environmentally-induced genes and mechanisms of inheritance: How are the effects of contaminant exposure transferred from one generation to the next?

- Articles in Refereed Scientific Journals:
  - Johnson, J., J. Silverstein, B. Small, W. R. Wolters, and B.S. Shepherd, In Press, Disparate Regulation of the Insulin-Like Growth Factor Binding Proteins in an Ictalurid Teleost (Ictalurus punctatus), General and Comparative Endocrinology.
  - Drennon, K., S. Moriyama, H. Kawauchi, B. Small, J. Silverstein, I. Parhar, and B. Shepherd, Accepted with Revision, Development of an Enzyme-Linked Immunosorbent Assay (ELISA) for the Measurement of Plasma Growth Hormone (GH) Levels in Channel Catfish (Ictalurus punctatus): Assessment of Environmental Salinity and GH-Secretagogues on Plasma GH Levels. General and Comparative Endocrinology.

Report Follows:

Problem and Research Objectives

The pituitary hormones, prolactin (PRL) and growth hormone (GH) are unequivocally involved in vertebrate development and the normal function of tissues and organs. Sex steroids, such as estrogen, control the development of the pituitary gland, its gene expression and the release of hormones (e.g., GH & PRL); however, the neuroendocrine system is further influenced by external factors such as stress, diet, physiological state, and pollutants which have been found to possess biological actions similar to that of estrogen (xenoestrogens). These hormonal mimetics are called "endocrine disrupting chemicals" (EDCs) or "xenoestrogens", because they possess estrogenic activities that can affect endogenous hormones in inappropriate ways. The idea that a single hormone (e.g., PRL) can regulate multiple physiological pathways, suggests that disturbances in its regulation, by an EDC, may negatively impact these pathways in an adult vertebrate and its offspring. We propose that contaminant exposure will not only affect pituitary physiology of the exposed organism, but will also influence the exposed organism's offspring through, as yet, poorly understood mechanisms. In this vein, scientists are increasingly using teleosts to study the effects of pollutants on vertebrate endocrinology as they are sensitive to these chemicals and encounter them routinely and chronically in their environment. To date, studies have mainly focused on the effects of EDCs on physiological end-points, with little emphasis on the impacted endocrine pathways themselves. Interestingly, recent reports suggest the presence of maternal mRNAs (hormone and hormone receptor mRNAs) in the unfertilized eggs of fish and this finding strongly suggests the involvement of these mRNAs in early embryonic development. Despite their presence, the significance and regulation of maternally-derived mRNAs have not been explored. Our aim was to study how estrogenic (e.g., hydroxylated PCBs) pollutants alter maternal endocrine physiology and how such alterations affect maternally-derived mRNAs in the unfertilized eggs of the Yellow Perch (Perca flavescens). An understanding of how maternal endocrine physiology affects offspring development will aid in future studies to improve the environmental monitoring and management practices of this important species in areas where endocrine disruption is suspected. To accomplish this, a major objective is the development of research tools that are needed to examine endocrine function in this, and other, important teleost species.

Methods

Using RT-PCR cloning procedures and DNA sequencing, we completed the cloning and characterization of the genes for several important hormones and are continuing to work on others. Total RNA was purified and first strand cDNAs were produced using 5' RACE techniques from pituitary (PRL, GH, SL) and liver (IGF-I) tissue. For cloning, primers were developed based upon conserved regions of known teleost sequences and PCR products were cloned into a plasmid and then transferred into competent *E. coli* cells. Cells were grown on LB agar with kanamycin to select for transformants and then specific clones were chosen, screened and grown in culture for plasmid isolation and DNA sequencing. We are conducting gene expression studies, using Northern Blotting, to identify the transcript size of the mRNAs for the hormones

that we have cloned as well as to determine tissue- and sex-specific differences in patterns of gene expression. In line with our efforts to develop methods to measure plasma hormone levels, we have some quantity of recombinant perch GH and antibody and plan to develop a radioimmunoassay that can be used to measure plasma GH levels in this teleost. We are also continuing our efforts to purify, through a new collaboration, other pituitary hormones (PRL & SL) important to growth and development in this teleost. Hormones will be sequentially purified using gel-filtration and HPLC procedures. Once putative hormone fractions for PRL and SL have been identified, antibodies will be developed in rabbits. We are also continuing field studies and have made significant strides in holding and maintaining yellow perch in our facilities at the University of Kentucky. We are also continuing to collect monthly samples of adult male and female yellow perch in order to examine sex- and season-specific changes in endocrine function and contaminant body burdens. In conjunction with our field studies, we are also sampling channel catfish as positive controls species. Our reason for this is that the perch and catfish inhabit different trophic niches. Furthermore, the catfish is an obligate, benthic-dwelling species, unlike the perch, and is therefore more likely to experience higher body burdens of environmental pollutants.

Principal Findings and Significance

At present, we have full-length cDNA clones for prolactin growth hormone, somatolactin, insulin-like growth factor-I and β-actin and partial clones for a newly identified hormone, termed "Ghrelin", which stimulates GH release and the β-estrogen receptor. Table 1 lists the nucleotide similarities of several other teleosts with the yellow perch PRL, GH, SL, and IGF-I cDNA sequences. Most notable is that yellow perch PRL appears to have a unique deviation from all other known teleost prolactins such that there is a codon gap associated with bases 190-192. In all but one of other known neo-teleost sequences, this codon encodes for the amino acid iso-leucine at position 64.

Table 1. Nucleotide sequence percent similarities for yellow perch PRL, GH, SL and IGF-I cDNAs against a taxonomically diverse group of teleosts. Dashes indicate that cDNA sequences were not available. An (*) designates the sequence with the greatest similarity as indicated by a standard nucleotide Blast search.

Yellow Perch	Black Sea Bream	Red Drum	Euro. Sea Bass	Sea Bream	Catfish	Zebra fish	Carp	Coho Salmon	Chum Salmon	Eel
PRL	-	-	86%*	86%	-	-	65%	-	70%	65%
GH	-	88%*	86%	87%	-	-	57%	63%	63%	-
SL	-	88%*	-	86%	59%	-	-	-	75%	64%
IGF-I	97%*	-	-	96%	-	68%	66%	83%	-	-

Also, in collaboration with Geoff Wallat (Ohio State Extension, Piketon, OH), we continue rearing larval perch in order to examine developmental-specific patterns of gene expression. This effort will allow us to identify and characterize the specific point at which females begin to grow faster than males and the endocrine basis for this difference.