

Division of Targeted Research

National Research Programmes

Wildhainweg 20

CH-3001 Berne

Tel. +41(0)31 308 22 22

Fax +41(0)31 305 29 70

E-Mail nfp@snf.ch

NRP Endocrine Disruptors

Intermediate Summary

Are organisational effects of estrogens on sexual differentiation development and growth of fish mediated via the insulin-like growth factor-I system?

Project leader

Prof. Dr. M. Reinecke

Project number

4050-66580

Potential interaction of (xeno)estrogens with the IGF-I system in fish growth and reproduction

The insulin-like growth factor I (IGF-I) system plays a central role in the complex system that regulates growth, differentiation, and reproduction. The present project investigates the hypothesis that the actions of estrogen(s) and xenoestrogen(s) on fish sexual differentiation and growth are partly mediated via IGF-I.

Project description:

Research questions

- As it gets increasingly important to deal with absolute amounts, particularly in endocrine disruptor research, we have developed a one-tube two-temperature real-time RT-PCR.
- The localization and expression of IGF-I mRNA (in situ hybridisation) and peptide (immunohistochemistry) in the male and female gonads of the tilapia (*Oreochromis mossambicus*) was determined at different developmental stages.
- The the localization and expression of IGF-I mRNA and peptide was analysed in (1) the liver as main source of circulating (endocrine) IGF-I, (2) tissues and organs involved in growth processes, such as cartilage and muscle, (3) organs involved in metabolism, such as the gastro-intestinal tract, exocrine and endocrine pancreas, (4) brain and pituitary as central regulators of the IGF-I and reproductive systems, and (5) in gills, kidney and heart as further models to study the impact of IGF-I in organogenesis
- To obtain insight into the potential interaction of E2 and IGF-I we performed an experiment in which developing tilapia were fed with an E2-enriched diet.

Results

Our newly established one-tube RT-PCR is highly sensitive (detection level ~ 2 pg/μg total RNA) and allows precise absolute quantification. The method is rapid as there are neither separate reverse transcriptions nor post-amplification steps, and can be executed with low risk of contamination. Currently, we use it to evaluate our estrogen exposure experiments.

The results of the study on the ontogeny of IGF-I in gonads and extragonadal sites showed:

- IGF-I appeared very early in ontogeny, i.e. around 4-5 days post fertilization (DPF), thus substantiating the assumed physiological impact of IGF-I in growth and organogenesis. IGF-I first appeared in tissues which are central to growth, such as liver (the main source of endocrine IGF-I), cartilage (chondrocytes and perichondrium), skeletal muscle and meninges.
- In brain, IGF-I mRNA and peptide occurred in the majority of neurones of larvae at early stages of development (from 5 to 29 DPF). However, in older larvae and adults IGF-I was present in a reduced number of neurones indicating that IGF-I seems to have a particular physiological impact in early brain development.
- In pituitary, IGF-I was also detected. The first neurosecretory axons containing IGF-I-immuno-reactivity appeared in the neuropituitary around 17 DPF. In endocrine cells of the adenopituitary IGF-I mRNA was detected at 20 DPF followed by IGF-I-immunoreactivity at 27 DPF.
- Both in males and females, IGF-I mRNA and peptide appeared first in somatic cells of the early gonad anlage. In the primordial germ cells only IGF-I peptide but no IGF-I mRNA was detected. Thus, IGF-I in the primordial germ cells seems to be of maternal origin. While in female germ cells IGF-I mRNA and peptide were first detected at 29 DPF, in male germ cells IGF-I appeared as late as at 47-48 DPF. Possibly, the appearance of IGF-I in the germ cells is linked to the onset of meiosis. According to results of the cooperating group of Dr. Barollier, in tilapia ovary the first meioses occur around 28 DPF and those in testes around 48-52 DPF.

In our E2 feeding experiment (125 μg E2/g food) of population of genetically male and female tilapia (n=200) we could rely on an established treatment protocol with a known outcome on sexual differentiation, in order to examine how the IGF system responds to a feminizing dose of E2. The effects of this treatment on developing tilapia were studied 1.5 months after completion of the treatment. The results of this E2-feeding study were:

- A significant feminisation of the treated population took place (82 % females versus 53 % females in the control group).
- The serum IGF-I level was decreased by 14% in the E2-treated group when compared to the untreated siblings even 1.5 months after end of the treatment.
- In correspondence, IGF-I gene expression in liver, the main source of circulating IGF-I, was significantly lowered (E2-treated males by about 66% and females in males by about 75%). No significant change in IGF-I mRNA occurred in the gills of female and male individuals.
- The amounts of IGF-I mRNA both in female and male gonads were reduced after E2-feeding by about 24% (female gonad) and by even 88% (male gonad). The latter reduction may be the cause for the impaired testes development.
- The most interesting result was a gender-specific opposite response of IGF-I expression in brain to the E2-treatment. In male brain there was a decrease of IGF-I mRNA by about 24% but in female brain IGF-I mRNA was increased by about 175%.

Perspectives

From the results obtained to date, we have information on the ontogeny of IGF-I in tilapia, and also preliminary evidence on an interaction between E2 and IGF-I. Particularly in brain and gonads a crosstalk of the hormone system seems to take place. Our study will be extended to IGF-II and the next research questions will address:

The spatial and temporal association of IGF-I, IGF-II, the aromatase levels and the estrogen receptors (ERs) in the hypothalamus-pituitary axis as well as in male and female gonads.